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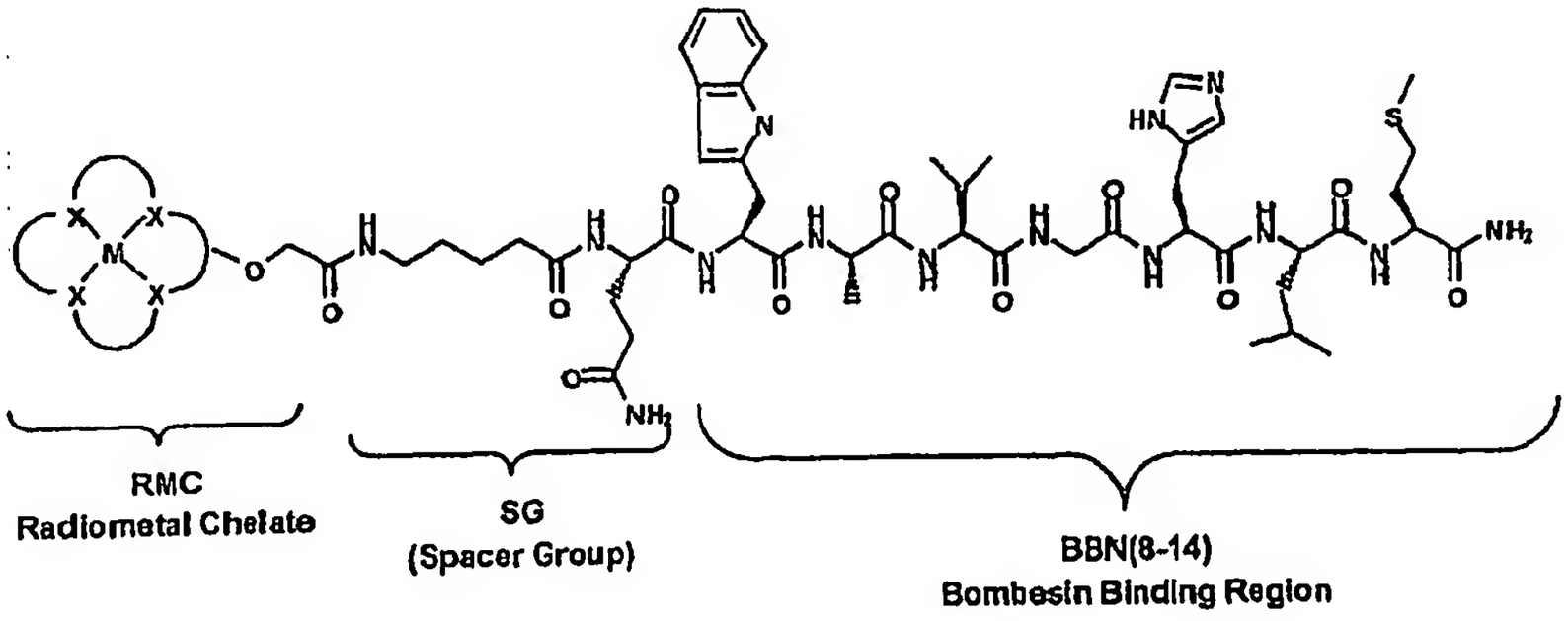
(54) Title: GASTRIN-RECEPTOR-AVID PEPTIDE CONJUGATES



(57) Abstract: A compound for use as a therapeutic or diagnostic radiopharmaceutical includes a group capable of complexing a medically useful metal attached to a moiety which is capable of binding to a gastrin releasing peptide receptor. A method for treating a subject having a neoplastic disease includes administering to the subject an effective amount of a radiopharmaceutical having a metal chelated with a chelating group attached to a moiety capable of binding to a gastrin releasing peptide receptor expressed on tumor cells with subsequent internalization inside of the cell. A method of forming a therapeutic or diagnostic compound includes reacting a metal synthon with a chelating group covalently linked with a moiety capable of binding a gastrin releasing peptide receptor.

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GASTRIN-RECEPTOR-AVID PEPTIDE CONJUGATES

TECHNICAL FIELD

This invention relates to radionuclide-labeled compounds useful as radiopharmaceuticals. More particularly, the present invention relates to conjugates of bombesin (BBN) analogues and a metal complexing group which, when complexed to a radionuclide, are useful as therapeutic and imaging agents for cancer cells that express gastrin releasing peptide (GRP) receptors.

BACKGROUND OF THE INVENTION

Detection and treatment of cancers using radiopharmaceuticals that selectively target cancers in human patients have been employed for several decades. Unfortunately, only a limited number of site-directed radiopharmaceuticals that exhibit highly specific *in vivo* localization in or near cancer cells are currently in routine use as being approved by the United States Food and Drug Administration (FDA). There is a great deal of interest in developing new radioactive drugs due to the emergence of more sophisticated biomolecular carriers that have high affinity and high specificity for *in vivo* targeting of tumors. Several types of agents are being developed and have been investigated including monoclonal antibodies (MAbs), antibody fragments (F_{AB}'s and $(F_{AB})_2$'s), and receptor-avid peptides [Bushbaum, 1995; Fischman et al., 1993; Schubiger et al., 1996].

The potential utility of using radiolabeled receptor-avid peptides for producing radiopharmaceuticals is best exemplified by ^{111}In-DTPA-conjugated octreotide (an FDA approved diagnostic imaging agent, Octreoscan®, marketed in the United States by Mallinckrodt Medical, Inc.) [Lowbertz et al. 1994]. This radiopharmaceutical is an ^{111}In-DTPA conjugate of Octreotide, a small peptide analogue of the human hormone somatostatin. This drug specifically binds to somatostatin receptors that are over-expressed on neuroendocrine cancers (e.g., carcinoid Ca, neuroblastoma, etc.) as well as others [Krenning et al., 1994]. Since ^{111}indium (^{111}In) is not the ideal

radionuclide for scintigraphic imaging, other somatostatin analogues and other receptor-avid biomolecules that are labeled with ^{99m}Tc (the optimal radionuclide for diagnostic imaging) are being studied and developed [Eckelman, 1995; Vallabhajosula et al., 1996].

Bombesin (BBN) is a 14 amino acid peptide that is an analogue of human gastrin releasing peptide (GRP) that binds to GRP receptors with high specificity and has an affinity similar to GRP [Davis et al., 1992]. GRP receptors have been shown to be over-expressed or uniquely expressed on several types of cancer cells. Binding of GRP receptor agonists (also autocrine factors) increases the rate of cell division of these cancer cells. For this reason, a great deal of work has been and is being pursued to develop BBN or GRP analogues that are antagonists [Davis et al., 1992; Hoffken, 1994; Moody et al., 1996; Coy et al., 1988; Cai et al., 1994]. These antagonists are designed to competitively inhibit endogenous GRP binding to GRP receptors and reduce the rate of cancer cell proliferation [Hoffken, 1994]. Treatment of cancers using these antagonists with these non-radioactive peptides requires chronic injection regimens (e.g., daily, using large quantities of the drug).

In designing an effective receptor-avid radiopharmaceutical for use as a diagnostic or therapeutic agent for cancer, it is important that the drug have appropriate *in vivo* targeting and pharmacokinetic properties [Fritzberg et al., 1992; Eckelman et al., 1993]. For example, it is essential that the radiolabeled receptor-avid peptide have high specific uptake by the cancer cells (e.g., via GRP receptors). In addition, it is necessary that once the radionuclide localizes at a tumor site, it must remain there for an extended time to deliver a highly localized radiation dose to the tumor. In order to achieve sufficiently high specific uptake of radiolabeled BBN analogues in tumors, the binding affinity of promising derivatives must be high (i.e., $K_d \cong$ 1-5 nmolar or less) with prolonged retention of radioactivity [Eckelman et al., 1995; Eckelman et al., 1993]. Work with ^{125}I-BBN derivatives has shown,

however, that for cancer cells that bind the ^{125}I-BBN derivatives (whether they be agonists or antagonists), the radioactivity is either washed off or expelled from the cells (*in vitro*) at a rapid rate [Hoffman et al., 1997]. Thus, these types of derivatives have a low probability of remaining "trapped" at the tumor site (*in vivo*) sufficiently long enough to be effective therapeutic or diagnostic agents.

Developing radiolabeled peptides that are cleared efficiently from normal tissues is also an important and especially critical factor for therapeutic agents. When labeling biomolecules (e.g., MAb, F_{AB}'s or peptides) with metallic radionuclides (via a chelate conjugation), a large percentage of the metallic radionuclide (in some chemical form) usually becomes "trapped" in either the kidney or liver parenchyma (i.e., is not excreted into the urine or bile) [Duncan et al., 1997; Mattes, 1995]. For the smaller radiolabeled biomolecules (i.e., peptides or F_{AB}'s), the major route of clearance of activity is through the kidneys, which in turn retain high levels of the radioactive metal (i.e., normally > 10-15% of the injected dose) [Duncan et al., 1997]. This presents a major problem that must be overcome in the development of therapeutic agents that incorporate metallic radionuclides. Otherwise, the radiation dose to the kidneys would be excessive. For example, ^{111}In-octreotide, the FDA approved diagnostic agent, exhibits high uptake and retention in kidneys of patients [Eckelman et al., 1995]. Even though the radiation dose to the kidneys is higher than desirable, it is tolerable in that it is a diagnostic radiopharmaceutical (it does not emit alpha- or beta-particles), and the renal dose does not produce observable radiation induced damage to the organ.

It has been found that conjugating non-metallated metal chelates to BBN derivatives can form GRP agonists that exhibit binding affinities to GRP receptors that are either similar to or approximately an order of magnitude lower than the parent BBN derivative. [Li et al.,1996a]. Recent results show that it is now possible to add radiometal chelates to BBN

analogues to form conjugates which are agonists and retain GRP receptor binding affinities that are sufficiently high (i.e., approximately 1-5 nmolar K_d's) for further development as potential radiopharmaceuticals. These agonist conjugates are transported intracellularly after binding to cell surface GRP receptors and are retained inside the cells for extended time periods. In addition, *in vivo* studies in normal mice have shown that retention of the radioactive metal in the kidneys was low (i.e., <5%), with the majority of the radioactivity excreted into the urine.

According to one aspect of the present invention, there is provided a BBN conjugate consisting of essentially a radio-metal chelate covalently appended to the receptor binding region of BBN [e.g., BBN(8-14) or BBN(7-14)] to form radiolabeled BBN analogues that have high specific binding affinities with GRP receptors. These analogues are retained for long periods of time inside GRP expressing cancer cells. Furthermore, their clearance from the bloodstream into the urine with minimal kidney retention is efficient. Preferably, the radiometals are selected from ^{99m}Tc, $^{186/188}Re$, ^{105}Rh, ^{153}Sm, ^{166}Ho, ^{90}Y, ^{199}Au, ^{177}Lu, ^{149}Pr, or ^{111}In, all of which hold the potential for diagnostic (i.e., ^{99m}Tc and ^{111}In) or therapeutic (i.e., $^{186/188}Re$, ^{105}Rh, ^{153}Sm, ^{166}Ho, ^{90}Y, ^{199}Au, ^{177}Lu, ^{149}Pm, ^{166}Dy, ^{175}Yb, ^{117m}Sm and ^{111}In) utility in cancer patients [Schubiger et al, 1996; Eckelman, 1995; Troutner, 1978].

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a compound for use as a therapeutic or diagnostic radiopharmaceutical, which includes a group that is capable of complexing a metal attached to a moiety capable of binding to a gastrin releasing peptide receptor.

Additionally, in accordance with the present invention, a method for treating a subject having a neoplastic disease includes the step of administering to the subject an effective amount of a radiopharmaceutical

having a metal chelated with a chelating group attached to a moiety capable of binding to a gastrin releasing peptide receptor on a cancer cell and being subsequently intracellularly transported and residualized inside the cell.

Additionally, in accordance with the present invention, a method of forming a therapeutic or diagnostic compound includes the step of reacting a metal synthon with a chelating group covalently linked with a moiety capable of binding a gastrin releasing peptide receptor.

BRIEF DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

FIGURE 1 illustrates a radiometal conjugate according to the present invention;

FIGURE 2 is an ORTEP drawing of the $\{Rh[16]aneS_4\text{-}olCl_2\}^+$, illustrating the crystal structure a Rh macrocycle;

FIGURE 3 illustrates a coupling reaction wherein a spacer group is coupled to a bombesin agonist binding moiety;

FIGURE 4 illustrates a coupling reaction for coupling a metal chelate to a peptide;

FIGURE 5 illustrates several iodinated bombesin analogues including their IC_{50}'s;

FIGURE 6 illustrates several tethered bombesin analogues;

FIGURE 7 illustrates several $[16]aneS_4$ bombesin analogues;

FIGURE 8 is a graph illustrating IC_{50} analysis wherein %-^{125}I-BBN total uptake versus molar concentration of displacing ligand is shown;

FIGURE 9 illustrates several Rh-[16]aneS_4 bombesin analogues;

FIGURE 10 illustrates an HPLC chromatogram of Rh-BBN-37 wherein (A) illustrates $^{105}RhCl_2$-BBN-37 and (B) illustrates $RhCl_2$-BBN-37;

FIGURE 11 is a graph illustrating ^{125}I-Tyr^4-bombesin internalization efflux from Swiss 3T3 cells;

FIGURE 12 illustrates ^{125}I bombesin internalization efflux in ^{125}I-free buffer wherein ^{125}I-Tyr^4-BBN vs. ^{125}I-Lys^3-BBN efflux from Swiss 3T3 cells is shown;

FIGURE 13 is a graph illustrating the efflux of ^{105}Rh-BBN-37 from Swiss 3T3 cells;

FIGURE 14 illustrates several ^{105}Rh bombesin analogues including their IC_{50}'s;

FIGURE 15 is a graph illustrating ^{105}Rh-BBN-61 efflux from Swiss 3T3 cells;

FIGURE 16 is a graph illustrating the efflux of ^{105}Rh-BBN-22 vs. ^{105}Rh-BBN-37 from Swiss 3T3 cells;

FIGURE 17 is two graphs illustrating Pancreatic CA cell binding wherein (A) illustrates the efflux ^{125}I-Tyr^4-BBN from CF PAC1 cells and (B) illustrates the efflux of ^{105}Rh-BBN-37 from CF PAC1 cells;

FIGURE 18 is two graphs illustrating Prostate CA cell binding wherein (A) illustrates the efflux of ^{125}I-Tyr^4-BBN from PC-3 cells and (B) illustrates the efflux of ^{105}Rh-BBN-37 from PC-3 cells;

FIGURE 19 illustrates five [16]aneS_4 bombesin analogues which utilize amino acids as Linking Groups;

FIGURE 20 illustrates four Rh-[16]aneS_4 bombesin analogues and IC_{50} values obtained in 3 cell lines;

FIGURE 21 illustrates three different N_3S-BFCA conjugates of BBN(7-14);

FIGURE 22 illustrates an HPLC chromatogram of ^{99m}Tc-BBN-122;

FIGURE 23 is a graph illustrating ^{99m}Tc-BBN-122 internalization into human prostate cancer cells (PC-3 cells);

FIGURE 24 is a graph illustrating ^{99m}Tc-BBN-122 internalization into human pancreatic tumor cells (CFPAC-1 cells);

FIGURE 25 is a graph illustrating ^{99m}Tc-RP-414-BBN-42 retention in PC-3 prostate cancer cells;

FIGURE 26 is a graph illustrating ^{99m}Tc-42 retention in CFPAC-1 pancreatic cancer cells;

FIGURE 27 illustrates further radiometal conjugates according to the present invention;

FIGURE 28 is three HPLC chromatograms of (A) DOTA-BBN[7-14-NH_2 (λ = 280 nm) (B) In-DOTA-BBN[7-14]NH_2 (λ = 280 nm) and (C) ^{111}In-DOTA-BBN[7-14]NH_2 (radiometric);

FIGURE 29 is a graph showing the competitive binding assay of In-DOTA-8-Aoc-BBN[7-14]NH_2 v. ^{125}I-Tyr^4-BBN in PC-3 cells;

FIGURE 30 is a graph showing the internalization of ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 in PC-3 cells;

FIGURE 31 is a graph showing the efflux of ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 in PC-3 cells; and

FIGURE 32 illustrates radiometal conjugate according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, compounds for use as diagnostic and/or therapeutic radiopharmaceuticals include a group capable of complexing a metal attached to a moiety capable of binding to a gastrin releasing peptide (GRP) receptor, as shown in Figure 1. These compounds

can be prepared with either a diagnostic radiometal or a therapeutic radiometal, thus affording utilities as either a diagnostic agent to identify cancerous tissues within the body using scintigraphic imaging techniques, or a therapeutic agent for the treatment and control of cancerous tissues. The moiety capable of specific binding to the GRP receptor is a GRP agonist. A GRP agonist activates or produces response by the GRP receptor upon interaction with the GRP receptor and is subsequently internalized inside of the cell by endocytosis. In contrast, a GRP antagonist counteracts the effect of an agonist and is not internalized inside of the cell.

More specifically, the GRP agonist for the purpose of this invention is a compound such as selected amino acid sequences or peptidomimetics, which are internalized or residualized following binding with high affinity and selectivity to GRP receptors and can be covalently linked to the metal complexing group. Many examples of specific modifications of the BBN(7-14) or BBN(8-14) that can be made to produce sequences with high antagonistic and agonistic binding affinity for GRP repectors have been reported by numerous investigations [Davis et al., 1992; Hoffken, 1994; Moody et al., 1996; Coy et al., 1988; Cai et al., 1994; Moody et al., 1995; Leban et al., 1994; Cai et al., 1992].

In a preferred embodiment of the present invention, the metal complexing group or moiety is a chelating agent or chelator which complexes to metals such as ^{105}Rh, $^{186/188}Re$, ^{99m}Tc, ^{153}Sm, ^{166}Ho, ^{90}Y, ^{111}In, ^{177}Lu, ^{149}Pm, ^{149}Sm or ^{199}Au. The chelating agent or chelator is attached or bound to the GRP agonist "binding region" through a spacer to produce a conjugate that retains its capability for high affinity and specific binding to GRP receptors.

In a more preferred embodiment of the present invention, the GRP agonist is a bombesin (BBN) analogue and/or a derivative thereof. The BBN derivative or analog thereof preferably contains either the same primary structure of the BBN binding region [i.e., BBN(8-14) or BBN(7-14)] or similar primary structures, with specific amino acid substitutions, that will specifically

bind to GRP receptors with better or similar binding affinities as BBN alone (i.e., $K_d \cong$ 1-5 nmolar). Compounds containing this BBN binding region (or binding moiety), when covalently linked to other groups (e.g., a radiometal chelate), are also referred to as BBN conjugates.

In general, the compounds of the present invention have a structure of the general formula:

X-Y-B

wherein X is a group capable of complexing a metal such as a radiometal; Y is a covalent bond or a spacer group; and B is a bombesin agonist binding moiety.

The metal bound to the metal complexing group can be any suitable metal chosen for a specific therapeutic or diagnostic use including transition metals, lanthanides, auger electron emitting isotopes, α, β or γ emitting isotopes. Preferably, the metal is a radiometal such as ^{105}Rh, ^{99m}Tc $^{186/188}Re$, ^{153}Sm, ^{166}Ho, ^{111}In, ^{90}Y, ^{177}Lu, ^{149}Pm, ^{153}Sm, and ^{199}Au whose chelates can be covalently linked (i.e., conjugated) to the specific BBN binding region via the N-terminal end of the primary binding sequence (e.g., BBN-8 or Trp^8) as shown in Figure 1.

In a preferred embodiment, the radiometal complexes are positioned by being spaced apart from or remotely from the amino acid Trp^8 by the spacer groups. The spacer groups can include a peptide (i.e., $\geq$ 1 amino acid in length), a hydrocarbon spacer of C_1-C_{10}, or a combination thereof. Preferably, the hydrocarbon spacer is a C_3-C_9 group. The resulting radiolabeled BBN conjugates retain high binding affinity and specificity for GRP receptors and are subsequently internalized inside of the cell.

The BBN conjugates can further incorporate a spacer group or component to couple the binding moiety to the metal chelator (or metal binding backbone) while not adversely affecting either the targeting function of the BBN-binding moiety or the metal complexing function of the metal chelating agent.

The term "spacer group" or "linker" refers to a chemical group that serves to couple the BBN binding moiety to the metal chelator while not adversely affecting either the targeting function of the BBN binding moiety or the metal complexing function of the metal chelator. Suitable spacer groups include peptides (i.e., amino acids linked together) alone, a non-peptide group (e.g., hydrocarbon chain), or a combination of an amino acid sequence and a non-peptide spacer. The types of spacer group used in most of the experimental studies described below in the Examples section were composed of a combination of L-glutamine and hydrocarbon spacers. A pure peptide spacer could consist of a series of amino acids (e.g., diglycine, triglycine, gly-gly-glu, gly-ser-gly, etc.), in which the total number of atoms between the N-terminal residue of the BBN binding moiety and the metal chelator in the polymeric chain is ≤ 12 atoms.

The spacer can also include a hydrocarbon chain [i.e., R_1-$(CH_2)_n$-R_2] wherein n is 0-10, preferably n = 3 to 9, R_1 is a group (e.g., H_2N-, HS-, -COOH) that can be used as a site for covalently linking the ligand backbone or the preformed metal chelator or metal complexing backbone; and R_2 is a group that is used for covalent coupling to the N-terminal NH_2-group of the BBN binding moiety (e.g., R_2 is an activated-COOH group). Several chemical methods for conjugating ligands (i.e., chelators) or preferred metal chelates to biomolecules have been well described in the literature [Wilbur, 1992; Parker, 1990; Hermanson, 1996; Frizberg et al., 1995]. One or more of these methods could be used to link either the uncomplexed ligand (chelator) or the radiometal chelate to the spacer group, or to link the spacer group to the BBN(8-14) derivatives. These methods include the formation of acid anhydrides, aldehydes, arylisothiocyanates, activated esters, or N-hydroxysuccinimides [Wilbur, 1992; Parker, 1990; Hermanson, 1996; Frizberg et al., 1995].

The term "metal complexing chelator" refers to a molecule that forms a complex with a metal atom that is stable under physiological

conditions. That is, the metal will remain complexed to the chelator backbone *in vivo*. More particularly, a metal complexing chelator is a molecule that complexes to a radionuclide metal to form a metal complex that is stable under physiological conditions and which also has at least one reactive functional group for conjugation with the BBN agonist binding moiety. Metal complexing chelators can include monodentate and polydentate chelators [Parker, 1990; Frizberg et al., 1995; Lister-James et al., 1997; Li et al., 1996b; Albert et al., 1991; Pollak et al., 1996; de Jong et al., 1997; Smith et al., 1997] and include the DOTA chelators discussed in more detail below. Metal complexing chelators include tetradentate metal chelators which can be macrocyclic and have a combination of four nitrogen and/or sulfur metal-coordinating atoms [Parker et al., 1990; Li et al., 1996b] and are designated as N_4, S_4, N_3S, N_2S_2, NS_3, etc., as shown in Figure 2. A number of suitable multidentate chelators that have been used to conjugate proteins and receptor-avid molecules have been reported [Frizberg et al., 1995; Lister-James et al., 1997; Li et al., 1996b; Albert et al., 1991; Pollak et al., 1996; de Jong et al., 1997] and include the DOTA chelators discussed in more detail below. These multidentate chelators can also incorporate other metal-coordinating atoms such as oxygen and phosphorous in various combinations. The metal binding complexing moiety can also include "3+1" chelators [Seifert et al., 1998].

For diagnostic purposes, metal complexing chelators preferably include chelator backbones for complexing the radionuclide metals ^{99m}Tc and ^{111}In. For therapeutic purposes, metal complexing chelators preferably include chelator backbones that complex the beta particle emitting radionuclide metals including ^{105}Rh, $^{186/188}$Re, ^{153}Sm, ^{90}Y, ^{166}Ho, ^{199}Au, ^{177}Lu, ^{111}In, ^{166}Dy, ^{175}Yb and ^{149}Pm [Schubiger et al., 1996; Hoffken, 1994].

As was briefly described above, the term "bombesin agonist" or "BBN agonist" refers to compounds that bind with high specificity and affinity to GRP receptors, and upon binding to the GRP receptor, are intracellularly

internalized. Suitable compounds include peptides, peptidomimetics and analogues and derivatives thereof. In particular, previous work has demonstrated that the region on the BBN peptide structure required for binding to GRP receptors spans from residue 8 through 14 [Davis et al., 1992; Hoffken, 1994; Moody et al., 1996; Coy, 1988; Cai et al., 1994]. The presence of methionine (Met) at position BBN-14 will generally confer agonistic properties while the absence of this residue at BBN-14 generally confers antagonistic properties [Hoffken, 1994].

It is well documented in the art that there are a few and selective number of specific amino acid substitutions in the BBN (8-14) binding region (e.g., D-Ala^{11} for L-Gly^{11} or D-Trp^{8} for L-Trp^{8}), which can be made without decreasing binding affinity [Leban et al., 1994; Qin et al., 1994; Jensen et al., 1993]. In addition, attachment of some amino acid chains or other groups to the N-terminal amine group at position BBN-8 (i.e., the Trp^{8} residue) can dramatically decrease the binding affinity of BBN analogues to GRP receptors [Davis et al., 1992; Hoffken, 1994; Moody et al., 1996; Coy, et al., 1988; Cai et al., 1994]. In a few cases, it is possible to append additional amino acids or chemical moieties without decreasing binding affinity. The effects of conjugating various side chains to BBN-8 on binding affinity, therefore, is not predicable.

The BBN conjugates of the present invention can be prepared by various methods depending upon the selected chelator. The peptide portion of the conjugate can be most conveniently prepared by techniques generally established and known in the art of peptide synthesis, such as the solid-phase peptide synthesis (SPPS) approach. Solid-phase peptide synthesis (SPPS) involves the stepwise addition of amino acid residues to a growing peptide chain that is linked to an insoluble support or matrix such as polystyrene. The C-terminal residue of the peptide is first anchored to a commercially available support with its amino group protected with an N-protecting agent such as a t-butyloxycarbonyl group (tBoc) or a

fluorenylmethoxycarbonyl (fmoc) group. The amino protecting group is removed with suitable deprotecting agents such as TFA in the case of tBOC or piperidine for fmoc and the next amino acid residue (in N-protected form) is added with a coupling agent such as dicyclocarbodiimide (DCC). Upon formation of a peptide bond, the reagents are washed from the support. After addition of the final residue, the peptide is cleaved from the support with a suitable reagent such as trifluoroacetic acid (TFA) or hydrogen fluoride (HF).

The spacer groups and chelator components are then coupled to form a conjugate by reacting the free amino group of the Trp^8 residue of the BBN binding moiety with an appropriate functional group of the chelator, metal chelator, or spacer group such as a carboxyl group or activated ester.

The BBN conjugate can also incorporate a metal complexing chelator backbone that is peptidic and compatible with solid-phase peptide synthesis. In this case, the chelator backbone can be added to the BBN binding moiety in the same manner as described above, or more conveniently, the metal complexing chelator backbone coupled to the BBN binding moiety can be synthesized *in toto* starting from the C-terminal residue of the peptide and ending with the N-terminal residue of the metal complexing chelator structure.

The chelator backbones used in accordance with the present invention are commercially available or they can, be made by methods similar to those outlined in the literature [Frizberg et al., 1995; Lister-James et al., 1997; Li et al., 1996b; Albert et al., 1991; Pollak et al., 1996; de Jong et al., 1997; Smith et al., 1997; Seifert et al., 1998]. Attachment of the spacer groups to functionalizable atoms appended to the ligand backbone can be performed by standard methods known to those skilled in the art. For example, the HOBt/HBTU activated -COOH group on 5-aminovaleric acid (5-AVA) was reacted with the N-terminal amine on Gln^7 to produce an amide linkage as shown in Figure 3. Similarly, the -COOH group attached to the characterized [16]aneS_4 ligand was conjugated to the amine group on the

hydrocarbon spacer (shown below) by reaction of the HOBt/HBTU activated carboxyl group appended to the [16]aneS_4 macrocycle with the terminal amine group on 5-AVA to form BBN-37 as shown in Figure 4. Other standard conjugation reactors that produce covalent linkages with amine groups can also be used [Wilbur, 1992; Parker, 1990].

The chelating framework, conjugated via Trp^8, that complexes the radiometals should form a 1:1 chelator to metal ratio. Since ^{99m}Tc has a short half-life (6 hours) and is a diagnostic radionuclide, the method of forming the ^{99m}Tc-BBN analogues should permit complexation (either directly or by transmetallation) of ^{99m}Tc to the conjugated chelating framework in a one-step, high yield reaction (exemplified below in the Experimental Section).

In contrast, the longer half-lives of the therapeutic radionuclides (e.g., ^{105}Rh, $^{186/188}$Re, ^{153}Sm, ^{166}Ho, ^{90}Y, ^{177}Lu, ^{149}Pm, ^{199}Au, ^{111}In, ^{177}Lu) permit formation of the corresponding radiolabeled BBN analogues by either a one-step, high yield complexation step, or by performing a ^{105}Rh, $^{186/188}$Re, ^{153}Sm, ^{166}Ho, ^{90}Y, ^{177}Lu, ^{111}In or ^{149}Au chelate synthon followed by conjugation of the preformed complex to the N-terminal end of the BBN binding moiety. In all cases, the resulting specific activity of the final radiolabeled BBN derivative must be high (i.e., > 1Ci/μmole).

Re- and Tc-conjugates

Re and Tc are both in row VIIB of the Periodic Table and they are chemical congeners. Thus, for the most part, the complexation chemistry of these two metals with ligand frameworks that exhibit high *in vitro* and *in vivo* stabilities are the same [Eckelman, 1995]. Many ^{99m}Tc or $^{186/188}$Re complexes, which are employed to form stable radiometal complexes with peptides and proteins, chelate these metals in their +5 oxidation state [Lister-James et al., 1997]. This oxidation state makes it possible to selectively place ^{99m}Tc- or $^{186/188}$Re into ligand frameworks already conjugated to the biomolecule, constructed from a variety of ^{99m}Tc(V) and/or $^{186/188}$Re(V) weak chelates (e.g., ^{99m}Tc- glucoheptonate, citrate, gluconate, etc.) [Eckelman,

1995; Lister-James et al., 1997; Pollak et al., 1996]. Tetradentate ligand frameworks have been shown to form well-defined, single chemical species in high specific activities when at least one thiol group or at least one hydroxymethylene phosphine group is present on the ligand backbone [Smith et al., 1997].

Ligands which form stable Tc(V) or Re(V) tetradentate complexes containing, but not limited to, amino N-atoms, amido-N-atoms, carboxy-O-atoms and thioether-S-atoms, are donor atoms that can also be present [Eckelman, 1995; Fritzberg et al., 1992; Parker, 1990; Frizberg et al., 1995; Pollak et al., 1996; Seifert et al., 1998]. Depending upon the mix of donor atoms (groups), the overall complex charge normally ranges from -1 to +1.

Incorporation of the metal within the conjugate can be achieved by various methods commonly known in the art of coordination chemistry. When the metal is ^{99m}technetium, the following general procedure can be used to form a technetium complex. A peptide-chelator conjugate solution is formed by initially dissolving the conjugate in water or in an aqueous alcohol such as ethanol. The solution is then degassed to remove oxygen. When an -SH group is present in the peptide, the thiol protecting group(s) are removed with a suitable reagent, for example with sodium hydroxide, and are then neutralized with an organic acid such as acetic acid (pH 6.0-6.5). In the labeling step, sodium pertechnetate obtained from a molybdenum generator is added to a solution of the conjugate with a sufficient amount of a reducing agent, such as stannous chloride, to reduce technetium, and is either allowed to stand at room temperature or is heated. The labeled conjugate can be separated from the contaminants $^{99m}TcO_4^-$ and colloidal $^{99m}TcO_2$ chromatographically, for example with a C_{18} Sep Pak cartridge [Millipore Corporation, Waters Chromatography Division, 34 Maple Street, Milford, Massachusetts 01757].

In an alternative method, the labeling can be accomplished by a transchelation reaction. The technetium source is a solution of technetium complexed with labile ligands facilitating ligand exchange with the selected chelator. Examples of suitable ligands for transchelation include tartrate, citrate, gluconate, and heptagluconate. It will be appreciated that the conjugate can be labeled using the techniques described above, or alternatively, the chelator itself may be labeled and subsequently coupled to the peptide to form the conjugate; a process referred to as the "prelabeled chelate" method.

When labeled with diagnostically and/or therapeutically useful metals, peptide-chelator conjugates or pharmaceutically acceptable salts, esters, amides, and prodrugs of the present invention can be used to treat and/or detect cancers, including tumors, by procedures established in the art of radiodiagnostics and radiotherapeutics [Bushbaum, 1995; Fischman et al., 1993; Schubiger et al., 1996; Lowbertz et al., 1994; Krønning et al., 1994]. A conjugate labeled with a radionuclide metal, such as ^{99m}technetium, can be administered to a mammal, including human patients or subjects, by intravenous or intraperitoneal injection in a pharmaceutically acceptable carrier and/or solution such as salt solutions like isotonic saline. The amount of labeled conjugate appropriate for administration is dependent upon the distribution profile of the chosen conjugate in the sense that a rapidly cleared conjugate may be administered in higher doses than one that clears less rapidly. Unit doses acceptable for ^{99m}Tc imaging radiopharmaceuticals are in the range of about 5-40 mCi for a 70kg individual. *In vivo* distribution and localization can be tracked by standard scintigraphic techniques at an appropriate time subsequent to administration; typically between 30 minutes and 180 minutes depending upon the rate of accumulation at the target site with respect to the rate of clearance at non-target tissue.

The compounds of the present invention can be administered to a patient alone or as part of a composition that contains other components

such as excipients, diluents, radical scavengers, stabilizers, and carriers, all of which are well known in the art. The compounds can be administered to patients either intravenously or intraperitoneally.

There are numerous advantages associated with the present invention. The compounds made in accordance with the present invention form stable, well-defined ^{99m}Tc or $^{186/188}Re$ conjugate analogues of BBN agonists. Similar BBN agonist analogues can also be made by using appropriate chelator frameworks for the respective radiometals to form stable well-defined products labeled with ^{153}Sm, ^{90}Y, ^{166}Ho, ^{105}Rh, ^{199}Au, ^{149}Pm, ^{177}Lu or ^{111}In. The radiolabeled BBN agonist conjugates selectively bind to neoplastic cells expressing GRP receptors, become internalized, and are retained in the tumor cells for extended time periods. Incorporating the spacer group between the metal chelator and the BBN agonist binding moiety maximizes the uptake and retention of the radioactive metal inside of the neoplasts or cancer cells. The radioactive material that does not reach (i.e., does not bind) the cancer cells is preferentially excreted efficiently into the urine with minimal radiometal retention in the kidneys.

Radiotherapeutics

The diagnostic application of these compounds can be as a first-line diagnostic screen for the presence of neoplastic cell using scintigraphic imaging, as an agent for targeting neoplastic tissue using handheld radiation detection instrumentation in the field of radioimmuno guided surgery (RIGS), as a means to obtain dosimetry data prior to administration of the matched pair therapeutic compound, and as a means to assess GRP receptor population as a function of treatment over time.

The therapeutic application of these compounds can be defined either as an agent that will be used as a first-line therapy in the treatment of cancer, as combination therapy where these radiolabeled agents could be utilized in conjunction with adjuvant chemotherapy, and as the matched pair therapeutic agent. The matched pair concept refers to one compound which

can serve as both a diagnostic and a therapeutic agent depending on the radiometal with the appropriate chelate selected, and can be understood in connection with the data set forth below.

Radioisotope therapy involves the administration of a radiolabeled compound in sufficient quantity to damage or destroy the targeted tissue. After administration of the compound (e.g., by intravenous or intraperitonal injection), the radiolabeled pharmaceutical localizes preferentially at the disease site (in this instance, tumor tissue that expresses the GRP-receptor). Once localized, the radiolabeled compound damages or destroys the diseased tissue with the energy that is released during the radioactive decay of the isotope that is administered.

The design of a successful radiotherapeutic involves several critical factors:

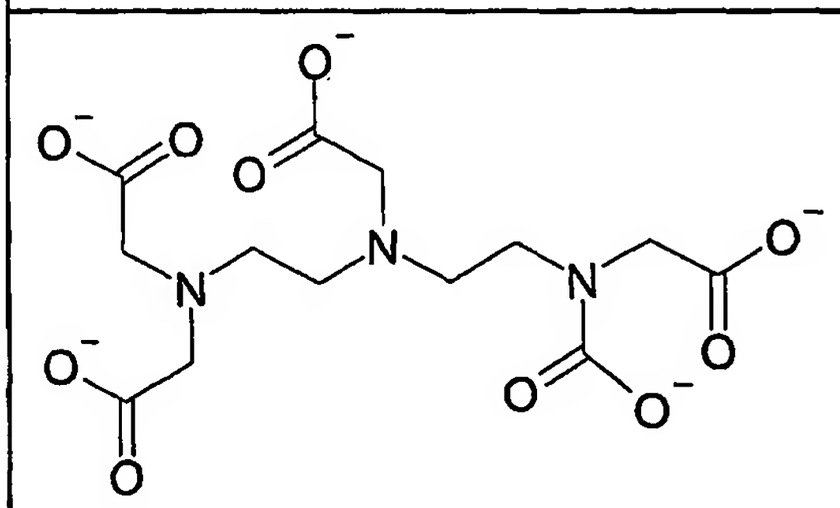
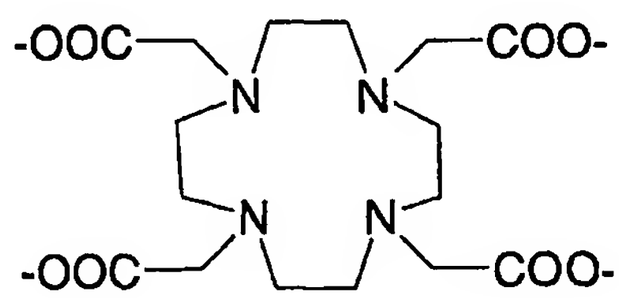
1. selection of an appropriate targeting group to deliver the radioactivity to the disease site;

2. selection of an appropriate radionuclide that releases sufficient energy to damage that the disease site, without substantially damaging adjacent normal tissues; and

3. selection of an appropriate combination of the targeting group and the radionuclide without adversely affecting the ability of this conjugate to localize at the disease site. For radiometals, this often involves a chelating group that coordinates tightly to the radionuclide, combined with a linker that couples said chelate to the targeting group, and that affects the overall biodistribution of the compound to maximize uptake in target tissues and minimizes uptake in normal, non-target organs.

The present invention provides radiotherapeutic agents that satisfy all three of the above criteria through proper selection of targeting group, radionuclide, metal chelate and linker.

Radiotherapeutic agents optionally contain a chelated+3 metal ion from the class of elements known as the lanthanides (elements of atomic number 57-71) and their analogs (i.e., M^{3+} metals such as yttrium and indium). Typical radioactive metals in this class include the isotopes ^{90}Yttrium, ^{111}Indium, ^{149}Promethium, ^{153}Samarium, ^{166}Dysprosium, ^{166}Holmium, ^{175}Ytterbium, and ^{177}Lu. All of these metals (and others in the lanthanide series) have very similar chemistries, in that they remain in the +3 oxidation state and prefer to chelate to ligands that bear hard (oxygen/nitrogen) donor atoms, as typified by derivatives of the well-known chelate DTPA (diethylenetriaminepentaacetic acid) and polyaza-polycarboxylate macrocycles such as DOTA (1,4,7,10-tetrazacyclododecane-N, N',N'',N'''-tetraacetic acid) and its close analogs. The structures of these chelating ligands in their fully deprotonated form are shown below.

DTPA	DOTA
	

These chelating ligands encapsulate the radiometal by binding to it via multiple nitrogen and oxygen atoms, thus preventing the release of free (unbound) radiometal into the body. This is important as *in vivo* dissociation of +3 radiometals from their chelate can result in uptake of the radiometal in the liver, bone and spleen [Brechbiel MW, Gansow OA, "Backbone-substituted DTPA ligands for ^{90}Y radioimmunotherapy, Bioconj. Chem. 1991; 2: 187-194; Li, WP, Ma DS, Higginbotham C, Hoffman T, Ketring AR, Cutler CS, Jurisson, SS, "Development of an *in vitro* model for assessing

the *in vivo* stability of lanthanide chelates," Nucl. Med. Biol. 2001; 28(2): 145-154; Kasokat T, Urich K. Arzneim-Forsch, "Quantification of dechelation of gadopentetate dimeglumine in rats", 1992; 42(6): 869-76]. Unless one is specifically targeting these organs, such non-specific uptake is highly undesirable as it leads to non-specific irradiation of non-target tissues, which can lead to such problems as hematopoietic suppression due to irradiation of bone marrow.

For radiotherapy applications, forms of the DOTA chelate [Tweedle MF, Gaughan GT, Hagan JT, "1-Substituted-1,4,7-triscarboxymethyl-1,4,7,10-tetraazacyclododecane and analogs" US Patent 4,885,363, Dec. 5, 1989] are particularly preferred, as the DOTA chelate is expected to dechelate less in the body than DTPA or other linear chelates.

General methods for coupling DOTA-type macrocycles to targeting groups through a linker (e.g., by activation of one of the carboxylates of the DOTA to form an active ester, which is then reacted with an amino group on the linker to form a stable amide bond), are known to those skilled in the art. (See e.g., Tweedle et al., US Patent 4,885,363). Coupling can also be performed on DOTA-type macrocycles that are modified on the backbone of the polyaza ring.

The selection of a proper nuclide for use in a particular radiotherapeutic application depends on many factors, including:

a. **Physical half-life** - This should be long enough to allow synthesis and purification of the radiotherapeutic construct from radiometal and conjugate, and delivery of said construct to the site of injection without significant radioactive decay prior to injection. Preferably, the radionuclide should have a physical half-life between about 0.5 and 8 days.

b. **Energy of the emission(s) from the radionuclide** - Radionuclides that are particle emitters (such as alpha emitters, beta emitters and Auger electron emitters) are particularly useful as they emit highly energetic particles that deposit their energy over short distances, thereby

producing highly localized damage. Beta emitting radionuclides are particularly preferred as the energy from beta particle emissions from these isotopes is deposited within 5 to about 150 cell diameters. Radiotherapeutic agents prepared from these nuclides are capable of killing diseased cells that are relatively close to their site of localization, but cannot travel long distances to damage adjacent normal tissue such as bone marrow.

c. **Specific activity (i.e. radioactivity per mass of the radionuclide)** - Radionuclides that have high specific activity (e.g., generator produced ^{90}Y, ^{111}In, ^{177}Lu) are particularly preferred. The specific activity of a radionuclide is determined by its method of production, the particular target that is used to produce it, and the properties of the isotope in question.

Many of the lanthanides and lanthanoids include radioisotopes that have nuclear properties that make them suitable for use as radiotherapeutic agents, as they emit beta particles. Some of these are listed in the table below.

Isotope	Half -Life (days)	Max □- energy (MeV)	Gamma energy (keV)	Approximate range of b-particle (cell diameters)
^{149}-Pm	2.21	1.1	286	60
153-Sm	1.93	0.69	103	30
166-Dy	3.40	0.40	82.5	15
166-Ho	1.12	1.8	80.6	117
175-Yb	4.19	0.47	396	
^{177}Lu	6.71	0.50	208	20
^{90}-Y	2.67	2.28	-	150

^{111}-In	2.810	Auger electron emitter	173, 247	< 5µm

Pm:promethium, Sm:samarium, Dy:dysprosium, Ho:holmium, Yb:ytterbium, Lu:lutetium, Y:yttrium, In:Indium

Methods for the preparation of radiometals, such as beta-emitting lanthanide radioisotopes are known to those skilled in the art and have been described elsewhere [e.g., Cutler CS, Smith CJ, Ehrhardt GJ.; Tyler TT, Jurisson SS, Deutsch E., "Current and potential therapeutic uses of lanthanide radioisotopes." Cancer Biother. Radiopharm. 2000; 15(6): 531-545]. Many of these isotopes can be produced in high yield for relatively low cost, and many (e.g., ^{90}Y, ^{149}Pm, ^{177}Lu) can be produced at close to carrier-free specific activities (i.e., the vast majority of atoms are radioactive). Since non-radioactive atoms can compete with their radioactive analogs for binding to receptors on the target tissue, the use of high specific activity radioisotope is important to allow delivery of as high a dose of radioactivity as possible to the target tissue.

Radiotherapeutic derivatives of the invention containing beta-emitting isotopes of rhenium (^{186}Re and ^{188}Re) are also particularly preferred.

Proper dose schedules for the radiotherapeutic compounds of the present invention are known to those skilled in the art. The compounds can be administered using many methods which include, but are not limited to, a single or multiple IV or IP injections, using a quantity of radioactivity that is sufficient to cause damage or ablation of the targeted GRP-R bearing tissue, but not so much that substantive damage is caused to non-target (normal) tissue. The quantity and dose required is different for different constructs, depending on the energy and half-life of the isotope used, the degree of uptake and clearance of the agent from the body, and the mass of the tumor. In general, doses can range from a single dose of about 30-50 mCi to a cumulative dose of up to about 3 Curies.

The radiotherapeutic compositions of the invention can include physiologically acceptable buffers and can require radiation stabilizers to prevent radiolytic damage to the compound prior to injection. Radiation stabilizers are known to those skilled in the art and may include, for example, para-aminobenzoic acid, ascorbic acid, gentistic acid and the like.

The following examples are presented to illustrate specific embodiments and demonstrate the utility of the present invention.

Experimental Section

EXAMPLE 1: Synthesis and *in vitro* binding assessment of synthetic BBN analogues employing hydrocarbon chain spacers

A. Synthesis:

Many BBN analogues were synthesized by Solid Phase Peptide Synthesis (SPPS). Each peptide was prepared by SPPS using an Applied Biosystems Model 432A peptide synthesizer. After cleavage of each BBN analogue from the resin using Trifluoracetic acid (TFA), the peptides were purified by C18 reversed-phase HPLC using a Vydac HS54 column and CH_3CN/H_20 containing 0.1% TFA as the mobile phase. After collection of the fraction containing the desired BBN peptide (approximately 80-90% yield in most cases), the solvent was evaporated. The identity of each BBN peptide was confirmed by FAB-mass spectrometry, Department of Chemistry - Washington University, St. Louis, MO.

Various amino acid sequences (in some cases including different chemical moieties) were conjugated to the N-terminal end of the BBN binding region (i.e., to BBN-8 or Trp^8). BBN analogue numbers 9, #15, #15i, 16, 16i and 18 were synthesized as examples of N-terminal modified peptides as shown in Figure 5.

Various tethered N-terminal (via Trp^8) BBN analogues were also synthesized by SPPS as exemplified by BBN-40, BBN-41, BBN-42, BBN-43,

BBN-44, BBN-45, and BBN-49 as shown in Figure 6. In these particular tethered peptides, a Glu residue was attached to Trp^8 followed by attachment of fmoc protected terminal amine groups separated from a -COOH group by 3-, 4-, 5-, 6-, 8- and 11-carbon chain (CH) spacers (Figure 6). These fmoc protected acids were added as the terminal step during the SPPS cycle. As described previously, each of the BBN analogues was purified by reversed-phase HPLC and characterized by high resolution mass spectroscopy. Peptide 49 employed only glutamine as the spacer group.

The [16]aneS_4 macrocyclic ligand was conjugated to selected tethered BBN analogues shown in Figure 6. The -OCH_2COOH group on the [16]aneS_4 macrocycle derivative was activated via HOBt/HBTU so that it efficiently formed an amide bond with the terminal NH_2 group on the spacer side arm (following deprotection). The corresponding [16]aneS_4 tethered BBN derivatives were produced and examples of four of these derivatives (i.e., BBN-22, -37, -46 and -47) are shown in Figure 7. As previously described, each [16]aneS_4 BBN derivative was purified by reversed-phase HPLC and characterized by FAB mass spectroscopy.

B. *In Vitro* Binding Affinities:

The binding affinities of the synthetic BBN derivatives were assessed for GRP receptors on Swiss 3T3 cells and, in some cases, on a variety of human cancer cell lines that express GRP receptors. The IC_{50} value of each derivative was determined relative to (i.e., in competition with) ^{125}I-Tyr^4-BBN (the Kd for ^{125}I-Tyr^4-BBN for GRP receptors in Swiss 3T3 cells is reported to be 1.6+0.4 nM) [Zueht et al., 1991]. The cell binding assay methods used to measure the IC_{50}'s is standard and used techniques previously reported [Jensen et al., 1993; Cai et al., 1994; Cai et al., 1992]. The methods used for determining IC_{50}'s for all GRP receptor binding compounds on all cell lines were similar. The specific method used to measure IC_{50}'s on Swiss 3T3 cells is briefly described as follows:

Swiss 3T3 mouse fibroblasts are grown to confluence in 48 well microtiter plates. An incubation media was prepared consisting of HEPES (11.916g/l), NaCl (7.598 g/l), KCl (0.574 g/l), $MgCl_2$ (1.106 g/l), EGTA (0.380 g/l), BSA (5.0 g/l), chymostatin (0.002 g/l), soybean trypsin inhibitor (0.200 g/l), and bacitracin (0.050 g/l). The growth media was removed, the cells were washed twice with incubation media, and incubation media was returned to the cells. ^{125}I-Tyr^4-BBN (0.01 uCi) was added to each well in the presence of increasing concentrations of the appropriate competitive peptide. Typical concentrations of displacing peptide ranged from 10^{-12} to 10^{-5} moles of displacing ligand per well. The cells were incubated at 37°C for forty minutes in a 95%O_2/5%CO_2 humidified environment. At forty minutes post initiation of the incubation, the medium was discarded, and the cells were washed twice with cold incubation media. The cells were harvested from the wells following incubation in a trypsin/EDTA solution for five minutes at 37°C. Subsequently, the radioactivity, per well, was determined and the maximum percent total uptake of the radiolabeled peptide was determined and normalized to 100%.

C. Results of Binding Affinity Measurements:

The IC_{50} values measured for the BBN derivatives synthesized in accordance with this invention showed that appending a peptide side chain and other moieties via the N-terminal BBN-8 residue (i.e., Trp^8) produced widely varying IC_{50} values. For example, see IC_{50} values shown for BBN 11, 15i, 16i, and 18 in Figures 5 and 8. The observations are consistent with previous reports showing highly variable IC_{50} values when derivatizing BBN(8-13) or BBN(8-14) with a predominantly short chain of amino acid residues [Hoffken, 1994]. In contrast, when a hydrocarbon spacer of 3- to 11-carbons was appended between BBN(7-14) and the [16]aneS_4 macrocycle, the IC_{50}'s were found to be surprisingly relatively constant and in the 1-5 nM range. The following IC_{50} values were obtained from the unmetallated compounds BBN-22, -37, -46, and –47 (structures shown in Figure 7).

COMPOUND	IC_{50} (nM)
BBN-22	3.01 ± 0.21
BBN-37	1.79 ± 0.09
BBN-46	2.34 ± 0.53
BBN-47	4.19 ± 0.91

These data suggest that using relatively simple spacer groups to extend ligands some distance from the BBN binding region [e.g., BBN(8-14)] can produce derivatives that maintain binding affinities in the 1-5 nmolar range.

D. Cell Binding Studies With Metal Complexes:

The following IC_{50} values were obtained for the metallated Rh complexes shown on Figure 9.

COMPOUND	IC_{50} (nM)
$RhCl_2$BBN-22	37.5 ± 10.5
$RhCl_2$BBN –37	4.76 ± 0.79
$RhCl_2$BBN –46	3.38 ± 0.69

The results illustrated in Figure 9 show that when the $RhCl_2$-[16]aneS_4 complexes separated from Trp^8 by only a glutamine (Glu^7), the IC_{50} of this conjugate (i.e., Rh-BBN-22) was 37.5 nM. However, when a five (5) carbon spacer or an eight (8) carbon spacer was present (i.e., Rh-BBN-37 and Rh-BBN-47), the IC_{50}'s remained below 5 nM. These data demonstrate that a straight chain spacer (along with Glu^7) to move the +1 charged Rh-S_4-chelate away from the BBN binding region, will result in a metallated BBN analogue with sufficiently high binding affinities to GRP receptors for *in vivo* tumor targeting applications.

E. ^{105}Radiolabeled BBN Analogues:

The ^{105}Rh conjugates of BBN-22, BBN-37, BBN-46 and BBN-47 were synthesized using a ^{105}Rh-chloride reagent from the Missouri University Research Reactor (MURR). This reagent was obtained as ^{105}Rh-chloride, a no-carrier-added (NCA) product, in 0.1-1.0M HCl. The pH of this reagent was

adjusted to 4-5 using 0.1-1.0M NaOH dropwise and it was added to approximately 0.1 mg of the [16]aneS_4-conjugated BBN derivatives in 0.9% aqueous NaCl and 10% ethanol. After the sample was heated at 80°C for one hour, the ^{105}Rh-BBN analogues were purified using HPLC. In each case, an NCA or high specific activity product was obtained since the non-metallated S_4-BBN conjugates eluted at a retention time well after the ^{105}Rh-BBN conjugates eluted. For example, the retention time of ^{105}Rh-BBN-37 was 7.1 minutes while BBN-37 eluted at 10.5 minutes from a C_{18}-reversed phase column eluted with CH_3CN/H_2O containing 0.1% TFA as shown in Figure 10A-B.

EXAMPLE 2: Retention of ^{105}Rh-BBN analogues in cancer cells

Once the radiometal has been specifically "delivered" to cancer cells (e.g., employing the BBN binding moiety that specifically targets GRP receptors on the cell surface), it is necessary that a large percentage of the "delivered" radioactive atoms remain associated with the cells for a period of time of hours or longer to make an effective radiopharmaceutical for effectively treating cancer. One way to achieve this association is to internalize the radiolabeled BBN conjugates within the cancer cell after binding to cell surface GRP receptors.

In the past, all of the work with synthetic-BBN analogues for treatment of cancers focused on synthesizing and evaluating antagonists [Davis et al., 1992; Hoffken, 1994; Moody et al., 1996; Coy et al., 1988; Cai et al., 1994; Moody et al., 1995; Leban et al., 1994; Cai et al., 1992]. After evaluating synthetic BBN analogues that would be predicted to be either agonists or antagonists, applicants found that derivatives of BBN(8-14) (i.e., those with the methionine or amidated methionine at BBN-14) are rapidly internalized (i.e., in less than two minutes) after binding to the cell surface GRP receptors. Several radiolabeled BBN(8-14) analogues that were studied to determine their internalization and intracellular trapping efficiencies were radioiodinated (i.e., ^{125}I) derivatives. The results of these studies

demonstrated that despite rapid internalization after ^{125}I-labeled BBN analogue binding to GRP receptors in Swiss 3T3 cells, the ^{125}I was rapidly expelled from the cells [Hoffman et al., 1997] as shown in Figure 11. Thus, these ^{125}I-BBN derivatives were not suitable for further development.

In contrast, the ^{105}Rh-BBN(8-14) derivatives that bind to GRP receptors are not only rapidly internalized, but there is a large percentage of the ^{105}Rh activity that remains trapped within the cells for hours (and in some cell lines greater than 24 hours). This observation indicates that these radiometallated BBN derivatives have real utility as radiopharmaceuticals for *in vivo* targeting of neoplasms expressing GRP receptors.

Experiments designed to determine the fraction of a radiotracer internalized within cells were performed by adding excess ^{125}I- or ^{105}Rh-BBN derivatives to the cell incubation medium. After establishment of equilibrium after a forty-minute incubation, the media surrounding the cells was removed and the cells were washed with fresh media containing no radioactivity. After washing, the quantity of radioactivity associated with the cells was determined (i.e., total counts per minute (TCPM) of ^{125}I or ^{105}Rh associated with the cells). The cells were then incubated in a 0.2M acetic acid solution (pH 2.5) which caused the surface proteins (including GRP receptors) to denature and release all surface-bound radioactive materials. After removing this buffer and washing, the cells were counted again. The counts per minute (c.p.m.) associated with the cells at that point were only related to the ^{125}I or ^{105}Rh that remained trapped inside of the cells.

To determine intracellular retention, a similar method was employed. However, after washing the cells with fresh (non-radioactive) incubation media, the cells were incubated in the fresh media at different time periods after washing away all extracellular ^{125}I- or ^{105}Rh-BBN analogues. After each time period, the methods used to determine TOTAL c.p.m. and intracellular c.p.m. after washing with a 0.2M acetic acid solution at pH 2.5 were the same as described above, and the percent ^{125}I or ^{105}Rh remaining

trapped inside of the cells was calculated. Figure 12 is a graph of results of efflux experiments using Swiss 3T3 cells with ^{125}I-Lys^3-BBN. The results show that there is rapid efflux of the ^{125}I from inside of these cells with less than 50% retained at fifteen minutes and by sixty minutes, less than 20% remained as shown in Figure 12.

In contrast, studies with all of the ^{105}Rh-[16]aneS$_4$-BBN agonist derivatives that are internalized inside of the cells showed substantial intracellular retention of ^{105}Rh by the GRP receptor expressing cells. For example, results of studies using ^{105}Rh-BBN-37 (see Figure 9) in conjunction with Swiss 3T3 cells showed that approximately 50% of the ^{105}Rh activity remains associated with the cells at sixty minutes post-washing, and approximately 30% of ^{105}Rh remained inside of the cells after four hours as shown in Figure 13. Note that at least 5% of the ^{105}Rh is surface bound at greater than or equal to sixty minutes.

The ^{105}Rh-BBN derivatives shown in Figure 9 all have an amidated methionine at position BBN-14 and are expected to be agonists [Jensen et al., 1993]. Therefore, they would be predicted to rapidly internalize after binding to GRP receptors on the cell surface [Reile et al., 1994; Bjisterbosch et al., 1995; Smythe et al., 1991], which was confirmed by Applicants' data. Referring to Figure 14, ^{105}Rh-BBN-61, a BBN analogue with no amino acid at position BBN-14 (i.e., a ^{105}Rh-BBN(8-13) derivative), was synthesized and studied. This BBN analogue has a high bonding affinity (i.e., IC_{50} = 4.1 nM). This type of derivative is expected to be an antagonist, and as such, will not internalize [Jensen et al., 1993; Smythe et al., 1991]. Results of efflux studies with ^{105}Rh-BBN-61 using Swiss 3T3 cells showed that immediately following washing with fresh incubation buffer (i.e., t=0), essentially all of the ^{105}Rh associated with these cells is on the cell surface, as expected. Furthermore, after only one hour of incubation, less than 10% remained associated with these cells in any fashion (comparing the results with the antagonist (see Figure 15) to those of the agonist (see Figure 16)).

These data indicate that ^{105}Rh-antagonists with structures similar to the ^{105}Rh-BBN agonists (i.e., those shown in Figure 9) are not good candidates for development of radiopharmaceuticals since they are neither trapped in nor on the GRP receptor expressing cells to nearly the same extent as the radiometallated BBN agonists.

EXAMPLE 3: Human Cancer Cell Line Studies

In vitro cell binding studies of ^{105}Rh-BBN-37 with two different human cancer cell lines that express GRP receptors (i.e., CF-PAC1 and PC-3 cell lines), which are tumor cells derived from patients with prostate CA and pancreatic CA, as shown in Figures 17A-B and 18A-B, respectively) were performed. Results of these studies demonstrated consistency with ^{105}Rh-BBN-37 binding and retention studies using Swiss 3T3 cells. Specifically, the binding affinity of Rh-BBN-37 was high (i.e., $IC_{50} \cong 7$ nM) with both human cancer cell lines as shown in Table 1. In addition, in all cells, the majority of the ^{105}Rh-BBN-37 was internalized and perhaps a major unexpected result was that the retention of the ^{105}Rh-tracer inside of the cells was significantly better than retention in Swiss 3T3 cells as shown in Figures 17 and 18. For example, it is particularly remarkable that the percentage of ^{105}Rh-BBN-37 that remained associated with both the CFPAC-1 and PC-3 cell line was greater than 80% at two hours after removing the extracellular activity by washing with fresh incubation buffer (see Figures 17 and 18).

EXAMPLE 4: *In Vivo* Studies

Biodistribution studies were performed by intravenous (I.V.) injection of either ^{105}Rh-BBN-22 or ^{105}Rh-BBN-37 into normal mice. In these studies, unanesthetized CF-1 mice (15-22g, body wt.) were injected I.V. via the tail vein with between one to five μCi (37-185 KBq) of the ^{105}Rh- labeled agent. Organs, body fluids and tissues were excised from animals sacrificed at 30, 60 and 120 minutes post-injection (PI). The tissues were weighed, washed in saline (when appropriate) and counted in a NaI well counter. These data were then used to determine the percent injected dose (% ID) in

an organ or fluid and the %ID per gram. The whole blood volume of each animal was estimated to be 6.5 percent of the body weight. Results of these studies are summarized in Tables 2 and 3.

Results from these studies showed that both the ^{105}Rh-BBN-22 and ^{105}Rh-BBN-37 were cleared from the bloodstream, predominantly via the kidney into the urine. Specifically, 68.4 ± 6.6% and 62.3 ± 5.8% of the ID was found in urine at two hours Pi of ^{105}Rh-BBN-22 and ^{105}Rh-BBN-37, respectively (see Tables 2 and 3). An unexpected finding was that the % ID of ^{105}Rh that remained deposited in the kidneys of these animals was only 2.4 ± 0.6 % ID and 4.6 ± 1.3 % ID at two hours post injection of ^{105}Rh-BBN-22 and ^{105}Rh-BBN-37 (see Tables 2 and 3). This is much less than would be expected from previously reported data where radiometallated peptides and small proteins have exhibited renal retention of the radiometal that is greater than 10% ID and usually much greater than 10% [Duncan et al., 1997]. The reason for reduced renal retention of ^{105}Rh-BBN analogues is not known, however, this result demonstrates a substantial improvement over existing radiometallated peptides.

Biodistribution studies also demonstrated another important *in vivo* property of these radiometallated BBN analogues. Both ^{105}Rh-BBN-22 and ^{105}Rh-BBN-37 are efficiently cleared from organs and tissues that do not express GRP receptors (or those that do not have their GRP receptors accessible to circulating blood). The biodistribution studies in mice demonstrated specific uptake of ^{105}Rh-BBN-22 and ^{105}Rh-BBN-37 in the pancreas while other non-excretory organs or tissues (i.e., heart, brain, lung, muscle, spleen) exhibited little or no uptake or retention (Tables 2 and 3). Both ^{105}Rh-BBN-22 and ^{105}Rh-BBN-37 were removed from the bloodstream by both the liver and kidneys with a large fraction of the ^{105}Rh removed by these routes being excreted into the intestines and the bladder, respectively. It is important to note that the % ID/gm in the pancreas of ^{105}Rh-BBN-22 and ^{105}Rh-BBN-37 was 3.9 $\pm$ 1.3 % and 9.9 $\pm$ 5.4 %, respectively at two hours, Pi.

Thus, the ratios of % ID/gm of ^{105}Rh-BBN-22 in the pancreas relative to muscle and blood were 16.2 and 7.6, respectively. The ratios of % ID/gm of ^{105}Rh-BBN-37 in the pancreas relative to muscle and blood were 25.4 and 29.1, respectively. These data demonstrated selective *in vivo* targeting of these radiometallated BBN analogues to cells expressing GRP receptors [Zhu et al., 1991; Qin et al., 1994] and efficient clearance from non-target tissues. If cancer cells that express GRP receptors are present in the body, these results indicate radiometallated BBN analogues will be able to target them with a selectivity similar to the pancreatic cells.

A comparison of the pancreatic uptake and retention of ^{105}Rh-BBN-22 with ^{105}Rh-BBN-37 demonstrated that ^{105}Rh-BBN-37 deposits in the pancreas with a two-fold better efficiency than ^{105}Rh-BBN-22 (i.e., 3.6 ± 1.2% ID and 2.3 ± 1.0 % ID) for ^{105}Rh-BBN-37 at one and two hours pi, respectively, versus 1.2 ± 0.5% ID and 1.0 ± 0.1% ID for ^{105}Rh-BBN-22 at one and two hours pi). This data is consistent with the greater than two-fold higher uptake and retention of ^{105}Rh-BBN- 37 found in the *in vitro* studies shown in Figure 16.

EXAMPLE 5: Synthesis and *in vitro* binding measurement of synthetic BBN conjugate analogues employing amino acid chain spacers

A. Synthesis:

Five BBN analogues were synthesized by SPPS in which between 2 to 6 amino acid spacer groups were inserted to separate a S_4-macrocyclic chelator from the N-terminal Trp^8 on BBN(8-14) (Figure 19). Each peptide was prepared by SPPS using an Applied Biosystems Model 432A peptide synthesizer. After cleavage of each BBN analogue from the resin using Trifluoracetic acid (TFA), the peptides were purified by C_{18} reversed-phase HPLC using a Vydac HS54 column and CH_3CN/H_20 containing 0.1% TFA as the mobile phase. After collection of the fraction containing the desired BBN peptide, the solvent was evaporated. The identity

of each BBN peptide was confirmed by FAB-mass spectrometry (Department of Chemistry - Washington University, St. Louis, MO).

Various amino acid sequences (in some cases containing different R-group moieties) were conjugated to the N-terminal end of the BBN binding region (i.e., to BBN-8 or Trp^8). BBN analogue numbers 96, 97, 98, 99 and 101 were synthesized as examples of N-terminal modified peptides in which the [16]aneS$_4$ macrocycle BFCA was separated from Trp^8 on BBN(8-14) by various amino acid sequences as shown in Figure 19.

The [16]aneS$_4$ macrocyclic ligand was conjugated to selected tethered BBN analogues. The -OCH$_2$COOH group on the [16]aneS$_4$ macrocycle derivative was activated via HOBt/HBTU so that it efficiently formed an amide bond with the terminal NH$_2$ group on the spacer side arm (following deprotection). The corresponding [16]aneS$_4$ tethered BBN derivatives were produced and examples of five of these derivatives (i.e., BBN-96, 97, 98, 99 and 101) are shown in Figure 19. As previously described, each [16]aneS$_4$ BBN derivative was purified by reversed-phase HPLC and characterized by FAB mass spectroscopy.

B. *In Vitro* Binding Affinities:

The binding affinities of the synthetic BBN derivatives were assessed for GRP receptors on Swiss 3T3 cells, PC-3 cells and CF PAC-1 cells. The IC$_{50}$'s of each derivative were determined relative to (i.e., in competition with) ^{125}I-Tyr^4-BBN. The cell binding assay method used to measure the IC$_{50}$'s is standard and was used by techniques previously reported [Jensen et al., 1993; Cai et al., 1992; Cai et al., 1994]. The methods used for determining IC$_{50}$'s with all BBN analogue binding to GRP receptors present on all three cell lines were similar. The specific method used to measure IC$_{50}$'s on Swiss 3T3 cells is briefly described as follows.

Swiss 3T3 mouse fibroblasts are grown to confluence in 48 well microliter plates. An incubation media was prepared consisting of HEPES (11.916g/l), NaCl (7.598 g/l), KCl (0.574 g/l), MgCl$_2$(1.106 g/l), EGTA (0.380

g/l), BSA (5.0 g/l), chymostatin (0.002 g/1), soybean trypsin inhibitor (0.200 g/1), and bacitracin (0.050 g/1). The growth media was removed, the cells were washed twice with incubation media, and incubation media was returned to the cells. ^{125}I-Tyr^4-BBN (0.01 μCi) was added to each well in the presence of increasing concentrations of the appropriate competitive peptide. Typical concentrations of displacing peptide ranged from 10^{-12} to 10^{-5} moles of displacing ligand per well. The cells were incubated at 37°C for forty minutes in a 95% O_2/5% CO_2 humidified environment. At forty minutes post initiation of the incubation, the medium was discarded, and the cells were washed twice with cold incubation media. The cells were harvested from the wells following incubation in a trypsin/EDTA solution for five minutes at 37°C. Subsequently, the radioactivity, per well, was determined and the maximum percent total uptake of the radiolabeled peptide was determined and normalized to 100%. A similar procedure was used in performing cell binding assays with both the PC-3 and CF_a-PAC-1 human cancer cell lines.

C. Results of Binding Affinity Measurements:

The IC_{50} values measured for the BBN derivatives synthesized in accordance with this invention showed that appending a chelator via amino acid chain spacer groups via the N-terminal BBN-8 residue (i.e., Trp^8) produced a variation of IC_{50} values. For example, see IC_{50} values shown for BBN 96, 97, 98 and 101 in Figure 19. The observations are consistent with previous reports showing variable IC_{50} values when derivatizing BBN(8-13) with a predominantly short chain of amino acid residues [Hoffken, 1994]. When the amino acid spacer groups used in BBN-98, 99 and 101 were appended between BBN(7-14) and the [16]aneS_4 macrocyle, the IC_{50}'s were found to be surprisingly constant and in the 1-6 nM range for all three cell lines (i.e., see IC_{50} values shown in Figure 19). These data suggest that using relatively simple spacer groups composed entirely of selected amino acid sequences to extend ligands some distance from the BBN region [e.g.,

BBN(8-14)] can produce derivatives that maintain binding affinities in the 1-6 nmolar range.

D. Cell Binding Studies with Rh-BBN-Conjugates:

Results illustrated in Figure 20 show that when the corresponding $RhCl_2$ [16]aneS_4 complex was separated from Trp^8 on BBN(8-14) by the four different amino acid spacer groups (see Figure 20), the IC_{50}'s of all four analogues (i.e., BBN-97, -98, -99, -101) were between 0.73 and 5.29 nmolar with GRP receptors on the PC-3 and CF PAC-1 cell lines. The IC_{50}'s for these same Rh-BBN conjugates were somewhat higher with the Swiss 3T3 cell line (Figure 20). These data demonstrate that amino acid chain with spacer groups used to move the +1 charged Rh-S_4 chelate away from the BBN binding region will result in a metallated BBN analogue with sufficiently high binding affinities to GRP receptors for *in vivo* tumor targeting applications.

EXAMPLE 6: Synthesis and *in vitro* binding assessment of a ^{99m}Tc-labeled synthetic BBN analogue

A. Synthesis:

Several tetradentate chelating frameworks have been used to form stable ^{99m}Tc or ^{188}Re labeled peptide and protein conjugates [Eckelman, 1995; Li et al., 1996b; Parker, 1990; Lister-James et al., 1997]. Many of these ligand systems contain at least one thiol (-SH) donor group to maximize rates of formation and stability (both *in vitro* and *in vivo*) of the resultant Tc(V) or Re(V) complexes [Parker, 1990; Eckelman, 1995]. Results from a recent report indicates that the bifunctional chelating agent (BFCA) dimethylglycyl-L-seryl-L-cyteinyl-glycinamide) (N_3S-BFCA) is capable of forming a well-defined complex with ReO^{+3} and TcO^{+3} [Wong et al., 1997]. Since this ligand framework can be synthesized by SPPS techniques, this N_3S-BFCA was selected for use in forming ^{99m}Tc-BBN-analogue conjugates. Three different N_3S-BFCA conjugates of BBN(7-14) were synthesized (BBN-120, -121 and -

122) as shown in Figure 21 by SPPS. BBN-120, BBN-121 and BBN-122 represent a series of analogues where the N_3S-BFCA is separated from the BBN(7-14) sequence by 3, 5, and 8 carbon spacer groups (Figure 21). Each peptide was synthesized and purified using the SPPS and chromatographic procedures outlined in Example 1. The thiol group on cysteine was protected using the ACM group, which is not cleaved during cleavage of these BBN-conjugates from the resin using TFA. The identities of BBN-120, -121 and -122 were confirmed by FAB mass spectrometry. Synthesis and purification of the N_3S-BFCA could also be readily accomplished using SPPS methods, followed by HPLC purification (see Example 1). The ACM group was used to protect the thiol group on cysteine during synthesis and cleavage from the resin.

B. *In Vitro* Binding Affinities:

Synthesis of ^{99m}Tc-BBN-122 (Figure 22) was achieved by two methods [i.e., (1) by transchelation of $^{99m}TcO^{+3}$ from ^{99m}Tc-gluconate; or (2) by formation of the "preformed" ^{99m}Tc-BFCA complex followed by -COOH activation with tetrafluorophenyl and subsequent reaction with the C_5-carbon spacer group appended to BBN(7-14)]. In both cases, the ^{99m}Tc-labeled peptide formed is shown in Figure 22. The structure of this Tc-BBN-122 conjugate was determined by using non-radioactive Re (the chemical congener of Tc). In these studies, the "preformed" ReO^{+3} complex with the N_3S-BFCA was prepared by reduction of ReO_4; with $SnCl_2$ in the presence of excess N_3S-BFCA dissolved in sodium phosphate buffered water at pH 6-6.5 by a method previously published [Wong et al., 1997]. After purification of the ReO-N_3S-BFCA complex, the structure of this chelate was shown (by massspectropy) to be identical to that previously reported [Wong et al., 1997].

The ReO-N_3-S-BFCA complex was converted to the activated trifluorophenyl (TFP) ester by adding 10 mg of the complex to 6 mg (dry) EDC and the 50 µl of TFP. After the solution was vortexed for one minute, CH_3CN was added until disappearance of cloudiness. The solution was incubated for

one hour at room temperature and purified by reversed-phase HPLC. To prepare the ReO-N_3S-BFCA complex BBN-122 conjugate (Figure 22), one µl microliter of the HPLC fraction containing the ReO-N_3S-BFCA complex was added to a solution containing one mg of the C_8-tethered BBN(7-14) peptide in 0.2 N $NaHCO_3$ at pH 9.0. After incubation of this solution for one hour at room temperature, the sample was analyzed and purified by reversed-phase HPLC. The yield of Re-BBN-122 was approximately 30-35%.

The method for preparation of the corresponding ^{99m}Tc-BBN-122 conjugate, using the "preformed" ^{99m}TcO-N_3S-BFCA complex, was the same as described above with the "preformed" ReO-N_3S-BFCA complex. In this case, $^{99m}TcO_4$, from a ^{99}Mo/^{99m}Tc generator was reduced with an aqueous saturated stannous tartrate solution in the presence of excess N_3S-BFCA. The yields of the ^{99m}Tc-BBN-122 product using this "preformed" method were approximately 30-40%. Reversed-phase HPLC analysis of the ^{99m}Tc-BBN-122, using the same gradient elution program[1] as used for analysis of the Re-BBN-122 conjugate, showed that both the ^{99m}Tc-BBN-122 and ^{188}Re-BBN-122 had the same retention time (i.e., 14.2-14.4 minutes) (See Figure 22). This provides strong evidence that the structures of both the ^{99m}Tc-BBN-122 and Re-BBN-122 are identical.

The binding affinities of BBN-122 and Re-BBN-122 were assessed for GRP receptors on Swiss 3T3 cells, PC-3 cells and CFPAC-1 cells that express GRP receptors. The $IC_{50's}$ of each derivative were determined relative to (i.e., in competition with) ^{125}I-Tyr^4-BBN (the Kd for ^{125}I-Tyr^4-BBN for GRP receptors in Swiss 3T3 cells is reported to be 1.6±0.4nM) [Zhu et al., 1991]. The cell binding assay methods used to measure the IC_{50}'s

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[1] Gradient elution program used in these studies was as follows.
Flow 1.5 ml/minute
Solvent A = HO with 0.1% TFA
Solvent B = CHCN with 0.1% TFA

Time (minutes)	%A/%B
0	95/5
25	30/70

are standard and were used according to techniques previously reported [Leban et al., 1994; Cai et al., 1994; Cai et al., 1992]. The methods used for determining IC_{50}'s with all GRP receptor binding of GRP receptors on all cell lines were similar and have been described previously for the other BBN-analogues and Rh-BBN analogues described in this document.

C. Results of Binding Affinity Measurements:

The IC_{50} values measured for BBN-122 and Re-BBN-122 synthesized in accordance with this invention showed that appending an 8-carbon hydrocarbon chain spacer linked to the N_3S1-BFCA and the corresponding Re complex (i.e., Trp^8) produced BBN conjugates with IC_{50} values in a 1-5 nmolar range (See Table A). When ^{99m}Tc-BBN-122 was incubated with these same cells, it was shown that greater than or equal nmolar concentrations of BBN displaced this ^{99m}Tc conjugate by more than 90%. This result demonstrates that ^{99m}Tc-BBN-122 has high and specific binding affinity for GRP receptors. These data suggest that using relatively simple spacer groups to extend the N_3S ligand framework and the corresponding Tc-or Re-N_3S_1, complexes some distance from the BBN binding region can produce derivatives that maintain binding affinities in the 1-5 nmolar range.

TABLE A.

Summary of IC_{50} values for GRP receptor binding for the non-metallated BBN-122 conjugate or the Re-BBN-122 conjugate in two cell lines (PC-3 and CF-PAC-1 cell lines that express GRP receptors). The IC_{50} values were measured using cell binding assays relative to ^{125}I-Tyr^4-BBN.

Conjugate	IC^{50} (nmolar)	
	PC-3	CF-PAC1
BBN-122	3.59 ± 0.75 (n=6)	5.58 ± 1.92 (n=14)

Re-BBN-122	1.23 ± 0.56 (n=12)	1.47 ± 0.11 (n=6)

EXAMPLE 7: Retention of ^{99m}Tc-BBN-122 in Human Cancer Cells PC-3 and CF-PAC-1 cells)

Once the radiometal has been specifically "delivered" to cancer cells (e.g., employing the BBN binding moiety that specifically targets GRP receptors on the cell surface), it is necessary that a large percentage of the "delivered" radioactive atoms remains associated with the cells for a period time of hours or longer to make an effective radiopharmaceutical for effectively treating cancer. One way to achieve this association is to internalize the radiolabeled BBN conjugates within the cancer cell after binding to cell surface GRP receptors.

Experiments designed to determine the fraction ^{99m}Tc-BBN-122 internalized within cells were performed by the same method previously described for ^{105}Rh-BBN-37. Briefly, excess ^{99m}Tc-BBN-122 was added to PC-3 or CFPAC-1 cell incubation media and allowed to establish equilibrium after a 40 minute incubation. The media surrounding the cells was removed and the cells were washed with fresh media containing no radioactivity. After washing, the quantity of radioactivity associated with the cells was determined (i.e., total counts per minute ^{99m}Tc associated with cells). The PC-3 and CFPAC-1 cells were then incubated in a 0.2M acetic acid solution (pH2.5), which caused the surface proteins (including GRP receptors) to denature and release all surface-bound radioactive materials. After removing this buffer and washing, the cells were counted again. The counts per minute (c.p.m.) associated with the cells at that point were only related to the ^{99m}Tc that remained trapped inside of the PC-3 or CFPAC-1 cells.

To determine intracellular retention of ^{99m}Tc activity, a similar method was employed. However, after washing the cells with fresh (non-

radioactive) incubation media, the cells were incubated in the fresh media at different time periods after washing away all extracellular ^{99m}Tc-BBN-122. After each time interval, the methods used to determine total c.p.m. and intracellular c.p.m. by washing with a 0.2M acetic acid solution at pH 2.5.

Studies with the ^{99m}Tc-BBN-122 agonist show that the agonist is internalized inside of the PC-3 and CFPAC-1 cells (Figures 23-26), and that substantial intracellular retention of ^{99m}Tc by the GRP receptor expressing cells occurs. For example, results of studies using ^{99m}Tc-BBN-122 in conjunction with PC-3 cells showed a high rate of internalization (Figure 23), and that approximately 75% of the ^{99m}Tc activity remains associated with the cells at 90 minutes post-washing (Figure 25). Almost all of this ^{99m}Tc cell-associated activity is inside of the PC-3 cells. Similar results were also found with the CFPAC-1 cells where there is also a high rate of ^{99m}Tc-BBN-122 internalization (Figure 24) and relatively slow efflux of ^{99m}Tc from the cells (i.e., 50-60% retention at 120 minutes post-washing) (Figure 26).

The ^{99m}Tc-BBN-122 peptide conjugate shown in Figure 22 has an amidated methionine at position BBN-14 and is expected to be an agonist [Jensen et al., 1993]. Therefore, it would be predicted to rapidly internalize after binding to GRP receptors on the cell surface [Bjisterbosch et al., 1995; Smythe et al., 1991], which is confirmed by Applicants' data in Figures 23-26.

EXAMPLE 8: *In Vivo* Studies.

Biodistribution studies were performed by intravenous (I.V.) injection of ^{99m}Tc-BBN-122 into normal mice. In these studies, unanesthetized CF-1 mice (15-22g, body weight) were injected I.V. via the tail vein with between one to five μCi (37-185 KBq) of ^{99m}Tc-BBN-122. Organs, body fluids and tissues were excised from animals sacrificed at 0.5, 1, 4 and 24 hours post-injection (pi). The tissues were weighed, washed in saline (when appropriate), and counted in a NaI well counter. These data were then used to determine the percent injected dose (% ID) in an organ or fluid and the % ID per gram. The whole blood volume of each animal was estimated to

be 6.5 percent of the body weight. Results of these studies are summarized in Tables B and C.

Results from these studies showed that ^{99m}Tc-BBN-122 is cleared from the bloodstream predominantly via the hepatobiliary pathway, showing about 35% of the ^{99m}Tc-activity cleared via the kidney into the urine. Specifically, 33.79±1.76% of the ID was found in urine at one hour post injection (Table B). The retention of ^{99m}Tc activity in the kidneys and liver is very low (Table B). This is much less than would be expected from previously reported data where radiometallated peptides and small proteins have exhibited renal retention of the radiometal that is great than 10% ID and usually much greater 10% [Duncan et al., 1997]. The reason for reduced renal retention of ^{99m}Tc-BBN-122 is not known; however, this result demonstrates a substantial improvement over existing radiometallated peptides.

Biodistribution studies also demonstrated another important *in vivo* property of ^{99m}Tc-BBN-122 in that it is efficiently cleared from organs and tissues that do not express GRP-receptors (or those that do not have their GRP-receptors accessible to circulating blood). The biodistribution studies in mice demonstrated specific uptake of ^{99m}Tc-BBN-122 in the pancreas while other non-excretory organs or tissues (i.e., heart, brain, lung, muscle, spleen) exhibited little or no uptake or retention. ^{99m}Tc-BBN-122 is removed from the bloodstream by both the liver and kidneys with a large fraction of the ^{99m}Tc removed by these routes being excreted into the intestines and the bladder, respectively. It is important to note that the percent ID/gm in the pancreas of ^{99m}Tc-BBN-122 is 12.63%/gm at 1 hour and drops to only 5.05% at the four hours post injection (Table C). Thus, the ratios of % ID/gm of ^{99m}Tc-BBN-122 in the pancreas relative to muscle and blood were 92.2 and 14.78 at four hours post injection, respectively. These data demonstrated selective *in vivo* targeting of this ^{99m}Tc-labeled BBN analogue to cells expressing GRP receptors [Zhu et al., 1991; Qin et al., 1994] and efficient clearance from non-

target tissues. If cancer cells that express GRP receptors are present in the body, these results indicate ^{99m}Tc-BBN analogues will be able to target them with a selectivity similar to the pancreatic cells.

EXAMPLE 9: Materials and Methods For Examples 9 and 10

The following abbreviations are used in the examples and derived from the following amino acids:

Ava = 5-amino valeric acid

Aoc = 8-amino octanoic acid

Aun = 11-amino undecanoic acid

Reagents and Apparatus: All chemicals were obtained from either Aldrich Chemicals (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). All chemicals and solvents used in these studies were reagent grade and used without further purification. The resin and fmoc-protected amino acids were purchased from Calbiochem-Novabiochem Corp (San Diego, CA) and the other peptide reagents from Applied Biosystems, Inc. (Foster City, CA). The DOTA-tris(t-butyl ester) was purchased from Macrocyclics (Dallas, TX) and the fmoc-protected w-amino alkyl carboxylic acids from Advanced ChemTech (Louisville, KY). ^{125}1-Tyr^4-Bombesin (^{125}I-Tyr^4-BBN) was obtained from NEN Life Sciences Products, Inc (Boston, MA). $^{111}InCl_3$ was obtained from Mallinckrodt Medical, Inc. (St. Louis, MO) as a 0.05N HCl solution. ^{90}Y was obtained from Perkin-Elmer (Biclerica, MA) as an HCl solution. Electrospray mass spectral analyses were performed by Synpep Corporation and T47D (Dublin, CA). Human prostate cancer PC-3 cells and MDA-MB-231 breast cancer cells were obtained from American Tissue Culture Collection (ATCC) and maintained and grown in the University of Missouri Cell and Immunology Core facilities. CF-1 mice were purchased from Charles River Laboratories (Wilmington, MA) and maintained in an in-house animal facility.

Solid Phase Peptide Synthesis (SPPS): Peptide synthesis was carried out on a Perkin Elmer - Applied Biosystems Model 432 automated peptide synthesizer employing traditional fmoc chemistry with HBTU

activation of carboxyl groups on the reactant with the N-terminal amino group on the growing peptide anchored via the C-terminus to the resin. Rink Amide MBHA resin (25 µmol), fmoc-protected amino acids with appropriate side-chain protections (7 µmol), fmoc-protected amino alkyl carboxylic acids (75 µmol), and DOTA-tris(t-butyl ester) (75 gmol) were used for the synthesis. The final products were cleaved by a standard procedure using a cocktail containing thioanisol, water, ethanedithiol and trifluoroacetic acid in a ratio of 2:1:1:36 and precipitated into methyl-t-butyl ether. Typical yields of the crude peptides were 80-85%. Crude peptides were purified by HPLC and the solvents were removed on a SpeedVac concentrator. The purified peptides were characterized by electrospray mass spectrometry. The mass spectral analysis results are shown in Table 4.

High performance liquid chromatography (HPLC): High performance liquid chromatography (HPLC) analyses for DOTA conjugates were performed on a Waters 600E system equipped with Varian 2550 variable absorption detector, Packard Radiometic 1 ^{50}TR flow scintillation analyzer, sodium iodide crystal radiometric detector, Eppendorf TC-50 column temperature controller, and Hewlett Packard HP3395 integrators. A Phenomenex Jupiter C_{-18} (5µm, 300 A0, 4.6 X 250 mm) column was used with a flow rate of 1.5 ml/minute. HPLC solvents consisted of H20 containing 0.1% trifluoroacetic acid (Solvent A) and acetonitrile containing 0.1% trifluoroacetic acid (Solvent B). HPLC gradient conditions for 0 spacer to 8- carbon spacer analogs begin with a solvent composition of 80% A and 20% B, followed by a linear gradient to 70%A:30% B over thirty minutes. HPLC gradient conditions for the 11-carbon spacer analysis are solvent composition of 75% A and 25% B, followed by a linear gradient to 50% A:50% B over thirty minutes.

Indium metallation: A solution of the unmetallated DOTA-BBN conjugates as shown in Table 4 (5.0 mg) in 0.2M tetramethylammonium acetate (0.5 ml) was added to indium trichloride ($InCl_3$) (10.0 mg). The pH of the reaction mixture was adjusted to 5.5 (Scheme 1). The reaction mixture

was incubated for one hour at 80°C. The resultant In-DOTA-BBN conjugate (Scheme 1) was purified by reversed-phase HPLC and analyzed by electrospray mass spectrometry. The mass spectral analysis results are shown in Table 4. The pure product was obtained as a white powder with a typical yield of 50-60%.

^{111}Indium/^{90}Yttrium labeling: An aliquot of $^{111}InCl_3$ (1.0 mCi, 50 µl) was added to a solution of the unmetallated DOTA-BBN (100 µg) conjugates shown in Table 4 in 0.2M tetramethyiammonium acetate (500 µl). The pH of the reaction mixture was adjusted to 5.6. The reaction mixture was incubated for one hour at 80°C. An aliquot of 0.002M EDTA (50 µl) was added to the reaction mixture to complex the unreacted $^{111}In^{+3}$. The resultant ^{111}In-DOTA-BBN conjugate was obtained as a single product and purified by reversed-phase HPLC. The purified ^{111}In-DOTA-BBN conjugate was then concentrated by passing through a 3M Empore C_{18} HD high performance extraction disk (7mm/3ml) cartridge and eluting with 33% ethanol in 0.1M NaH_2PO_4 buffer (400 µl). The concentrated fraction was then diluted with 0.1M NaH_2PO_4 buffer (2.3 ml, pH-7) to make the final concentration of ethanol in the solution <5%. The ^{90}Y DOTA-BBN complex was similarly prepared.

In Vitro Cell Binding Studies: The IC_{50} of the various In-DOTA-BBN conjugates was determined by a competitive displacement cell binding assay using ^{125}I-Tyr^4-BBN. Briefly, 3 X 104 cells suspended in RPMI medium 1640 at pH-7.4 containing 4.8 mg/ml HEPES, 0.1 µg/ml Bacitracin and 2 mg/ml BSA, were incubated at 37°C and a 5% CO_2 atmosphere for 40 minutes in the presence of 20,000 cpm ^{125}I-Tyr^4-BBN and increasing concentration of the In-DOTA-BBN conjugates. After the incubation, the reaction medium was aspirated and cells were washed four times with media. The radioactivity bound to the cells was counted in a Packard Riastar gamma counting system. The percent ^{125}I-Tyr^4-BBN bound to cells was plotted versus increasing concentrations of In-DOTA-BBN conjugates to determine the respective IC_{50} values.

Internalization and efflux studies: *In vitro* studies to determine the degree of internalization of the ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 conjugate were carried out by a method similar to that described by Rogers, et al. These studies were performed by incubating 3 X 104 cells suspended in RPMI medium 1640 at pH-7.4 containing 4.8 mg/ml HEPES, 0.1 µg/ml Bacitracin and 2 mg/ml BSA, at 37°C and a 5% CO_2 atmosphere for 40 minutes in the presence of 20,000 cpm ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 conjugate. After the incubation, the reaction medium was aspirated and cells were washed with media. The percent of cell-associated activity as a function of time (in the incubating medium at 37°C) was determined. The percentage radioactivity trapped in the cells was determined after removing activity bound to the surface of the cells by washing with a pH-2.5 (0.2M acetic acid and 0.5M NaCl) buffer one, two, three and four hours afterwards.

In vivo pharmacokinetic studies in CF-1 mice: The biodistribution and uptake of ^{111}In-DOTA-BBN conjugates in CF-1 mice was studied. The mice (average weight, 25 g) were injected with aliquots (50-100 µl) of the labeled peptide solution (55-75 kBq) in each animal via the tail vein. Tissues and organs were excised from the animals sacrificed at one hour post-injection. The activity counted in a NaI counter and the percent injected dose per organ and the percent injected dose per gram were calculated. The percent injected dose (% ID) in the blood was estimated assuming a blood volume equal to 6.5% of the total body weight. Receptor blocking studies were also carried out where excess (100 µg) BBN was administered to animals along with the ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2.

In vivo pharmacokinetic studies in human tumor bearing SCID mice: The biodistribution studies of the ^{111}In and ^{90}Y conjugates were determined in SCID mice bearing human tumor xenografts of either PC-3 (human androgen independent prostate cancer cell origin), or MDA-MB-231 (human breast cancer cell origin) cell lines. The xenograft models were produced by bilateral flank inoculation of 5 X 106 cells (PC-3 or MDA-MB-231

cells) per site. Four to six weeks post inoculation, palpable tumors were observed. At this point, the mice were injected with 4μCi of the complex in 100/μL of isotonic saline via the tail vein. The mice were euthanized and tissues and organs were excised from the animals at selected times post-injection (p.i.), including 15 minutes, 30 minutes, 1 hour, 4 hours, 24 hours, 48 hours, and 72 hours p.i. Subsequently, the tissues and organs were weighed and counted in a NaI well counter, and the percent injected dose (%ID) and %ID/g of each organ or tissue calculated. The %ID in whole blood was estimated assuming a whole-blood volume of 6.5% total body weight.

In vivo pre-clinical evaluation of single dose radiotherapy in human tumor bearing SCID mice: Pre-clinical therapeutic evaluation of the ^{90}Y-DOTA-8-Aoc-BBN[7-14]NH_2 conjugate was performed in SCID mice bearing PC-3 human androgen independent prostate cancer cell human tumor xenografts. The xenograft model was produced by bilateral flank inoculation of 5 X 106 PC-3 cells per site. Twenty-one days post inoculation, when palpable tumors appeared, single dose administration of ^{90}Y-DOTA-8-Aoc-BBN[7-14]NH_2 was initiated. Baseline weights, hematology profiles, and tumor measurements were obtained immediately prior to therapy administration. Four groups of animals were utilized: a saline placebo, a 5 mCi/kg single dose, a 10 mCi/kg single dose, and a 20 mCi/kg single dose. Tumor measurements and weights were obtained twice weekly throughout the 14 weeks post injection.

Discussion

A series of BBN-agonists containing the DOTA chelation system separated by spacers have been synthesized and characterized [Figures 27 and 28; Tables 4 and 20].

The *in vitro* binding affinity of the Indium-BBN analogs was measured in two cell lines: the human prostate cancer cell line, PC-3; and the human breast cancer cell line, T47D. Of the compounds tested, optimum

binding of the In-DOTA-8-Aoc-BBN[7-14]NH_2 analog was demonstrated in both cell lines examined [Figure 29 and Table 5].

The In-DOTA-8-Aoc-BBN[7-14]NH_2 analog underwent rapid receptor mediated endocytosis using an *in vitro* PC-3 cell assay system. Once internalized within PC-3 cells, the In-DOTA-8-Aoc-BBN[7-14]NH_2 analog remained retained within the cells for a prolonged time period [Figures 30 and 31].

In vivo analysis of the DOTA-BBN analogs in CF1 mice demonstrates that ^{111}In-DOTA-5-Ava-BBN[7-14]NH_2, ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2, ^{111}In-DOTA-11-Aun-BBN[7-14]NH_2 all target GRP receptor expression *in vivo* based on high uptake and accumulation of these compounds in the normal pancreas. Increasing hydrocarbon spacer length linking the DOTA metal chelation moiety to the GRP receptor binding moiety (BBN[7-14]NH_2), results in compounds with increased hydrophobicity, which subsequently shifts the clearance of these agents, from the renal system (hydrophilic agents) to the hepatobiliary system (hydrophobic agents). [Tables 6 and 7].

Specific *in vivo* GRP receptor binding was demonstrated by performing competitive blocking assays in CF1 normal mice, where greater than 98% of the normal receptor mediated uptake of ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 in normal pancreatic tissue was blocked by co-administration of an excess of bombesin [Tables 8 and 9].

Prolonged PC-3 human prostate tumor uptake was demonstrated for ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 and ^{90}Y -DOTA-8-Aoc-BBN[7-14]NH_2 using a xenograft mouse model [Tables 10-13].

Prolonged MDA-MB-23 1 human breast tumor uptake was demonstrated for ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 using a xenograft mouse model. [Table 14].

These *in vivo* pharmacokinetic studies in CF1 mice have demonstrated that the radiometallated bombesin analogues (^{111}In and ^{90}Y) clear from the blood pool into the renal-urinary excretion pathway.

Competitive Binding Assay Results

The ^{111}In and ^{90}Y complexes of one lead candidate, DOTA-8-Aoc-BBN[7-14]NH_2, have been synthesized and evaluated *in vitro* and *in vivo*. *In vitro* competitive binding assays, employing PC-3 human prostate tumor cells, demonstrated an average IC_{50} value of 1.69 nM for the In-DOTA-8-Aoc-BBN[7-14]NH_2 complex.

In Vivo Pharmacokinetic Results in PC-3 Tumor Bearing Mice

In vivo pharmacokinetic studies of ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 in PC-3 prostate tumor bearing mice conducted at 1, 4, 24, 48, and 72 hours p.i. revealed efficient clearance from the blood pool (0.92 ± 0.58 % ID, 1 hour p.i.) with excretion through the renal and hepatobiliary pathways (87% ID and 8.5% ID, at 24 hours p.i., respectively). Similar pharmacokinetic properties were observed with ^{90}Y-DOTA-8-Aoc-BBN[7-14]NH_2. Tumor targeting of PC-3 xenografted SCID mice resulted in tumor uptake and retention values of 3.63 ± 1.11%ID/g, 1.78 ± 1.09%ID/g, and 1.56 ± 0.45%ID/g obtained at 1,4, and 24 hours p.i. respectively, for the ^{111}In-DOTA-8-Aoc-BBN-[7-14]NH_2 complex. ^{90}Y-DOTA-8-Aoc-BBN[7-14]NH_2 exhibited nearly identical PC-3 tumor uptake and retention values of 2.95 ± 0.99%ID/g, 1.98 ± 0.66%ID/g, and 1.08 ± 0.37%ID/g at 1, 4, and 24 hours p.i., respectively. Initial therapeutic assessment of the ^{90}Y complex in PC-3 xenografted mice demonstrated that radiation doses of up to 20mCi/kg were well tolerated with overall survival exhibiting a dose dependent response.

These pre-clinical observations show that peptide conjugates of this type exhibit properties suitable as clinical therapeutic/diagnostic pharmaceuticals.

EXAMPLE 10: Binding of DOTA-BBN Conjugates in Human Breast Cancer Cell Lines:

Expression of Gastrin Releasing Peptide receptors (GRP-Rs) in a variety of cancers including breast, prostate, small cell lung, and pancreatic is well known. Recently, the first positive clinical images of GRP-R expression in human metastatic breast cancer patients were obtained [C. Van de Wiele et al., Eur. J. Nucl. Med. (2000) 27:1694-1699] with the compound, ^{99m}Tc-N_3S-5-Ava-BBN(7-14)NH_2, initially developed in our laboratory. The continued efforts in the development of GRP targeted radiopharmaceuticals have led to the synthesis of a series of DOTA incorporated peptides for the complexation of ^{111}In/^{90}Y.

Methods: Six synthetic peptides were constructed in an X-Y-Z fashion where X = the DOTA chelation system, Y = the linking arm, and Z = the BBN(7-14)NH_2 sequence. The six peptides differed in the selection of linking arms, comprising either amino acid tethers: -G-G-G-, -G-S-G- or S-G-S-, or alkyl carbon chain tethers; 5-Ava, 8-Aoc, or 11-Aun. The In complexes of all peptides were prepared, purified by RPHPLC, and characterized by ES-MS as described in Example 9.

Results: Pharmacokinetic studies conducted in CF1 mice revealed that ^{111}In-DOTA-8-Aoc-BBN(7-14)NH_2 exhibited optimum clearance kinetics while maintaining selective and high *in vivo* GRP receptor targeting. ^{111}In-DOTA-8-Aoc-BBN(7-14)NH_2 exhibited an IC_{50} value of 1.23 ±0.25nM for the GRP receptor expressed by the T47D human breast cancer cell line. Pharmacokinetic studies of ^{111}In-DOTA-8-Aoc-BBN(7-14)NH_2 conducted in MDA-MB-231 human breast cancer cell line xenografted SCID mice demonstrated specific tumor targeting with 0.83 ± 0.23% ID/g obtained at one hour post injection. Residualization of the radiolabel within the tumor was observed with 46% and 28% of the initial uptake retained at 4, and 24 hours, respectively.

Conclusion: These results show that GRP-R specific radiopharmaceuticals incorporating the DOTA chelation system are beneficial

for the development of diagnostic/therapeutic matched pair agents to target breast cancer.

EXAMPLE 11: Lutetium DOTA-BBN Compounds

A conjugate, ^{177}Lu-DOTA-8-Aoc-BBN[7-14]NH_2, was routinely prepared in high yield (≥95%) by addition of $^{177}LuCl_3$ to an aqueous solution (Ammonium Acetate) of DOTA-8-Aoc-BBN[7-14]NH_2 (3.4x10-8 mols) [pH = 5.5, Temp. = 800C, RT = 1 hour]. RCP determination demonstrated the stability of the conjugate over a wide range of pH values over a time course of 24 hours. The HPLC chromatogram of ^{177}Lu-DOTA-8-Aoc-BBN[7-14]NH_2 showed a retention time of 19.0 minutes. Under identical chromatographic conditions, DOTA-8-Aoc-BBN[7-14]NH_2 has a retention time of 20.5 minutes, allowing for peak purification of the radiolabeled conjugate. Collection of and counting of the ^{177}Lu-DOTA-8-Aoc-BBN[7-14]NH_2 eluant peak in a NaI well counter further demonstrated the stability of the new complex, as ≥95% of the activity loaded onto the column was recovered as a singular species.

The biodistribution studies of ^{177}Lu-DOTA-8-Aoc-BBN[7-14]NH_2 were determined in tumor bearing (PC-3) SCID mice (TABLE 19). This ^{177}Lu-conjugate cleared efficiently from the bloodstream within one hour post-injection. For example, 0.62 ± 0.44%ID remained in whole blood at one hour p.i. The majority of the activity was excreted via the renal-urinary excretion pathway (i.e., 67.41 ± 2.45% at one hour p.i. and 85.9 ± 1.4% at 24 hour p.i.), with the remainder of the radioactivity being excreted through the hepatobiliary pathway. Receptor-mediated, tumor targeting of the PC-3 xenografted SCID mice resulted in tumor uptake and retention values of 4.22 ± 1.09%ID/g, 3.03 ± 0.91%ID/g, and 1.54 ± 1.14%ID/g at 1, 4, and 24 hours, respectively.

EXPERIMENTAL: To 50µg (3.4x10-8 mols) of DOTA-8-Aoc-BBN[7-14]NH_2 in 50µL of 0.2M Ammonium Acetate was added 150µL of 0.4M Ammonium Acetate. To this solution was added 50µL of $^{177}LuCl_3$ (2mCi in

0.05N HCl, Missouri University Research Reactor). The solution was allowed to incubate at 800C for one hour, after which 50μg of 0.002M EDTA was added in order to scavenge uncomplexed Lutetium. Quality control of the final product was determined by reversed-phase HPLC. Peak purification of the labeled species was performed by collecting the sample from the HPLC eluant, into a solution of 1mg/mL bovine serum albumin/0.1M Na_2HPO_4. All further analyses were carried out using the HPLC-purified products.

HPLC analysis of each of the new compounds was performed using an analytical C_{18} reversed phase column (Phenomenex, 250x4.6mm, 5μm). The mobile phase consisted of a linear gradient system, with solvent A corresponding to 100% water with 0.1% trifluoroacetic acid, and solvent B corresponding to 100% acetonitrile with 0.1% trifluoroacetic acid. The mobile phase started with solvent compositions of 80%A:20%B. At time = 30 minutes, the solvent compositions were 70%A:30%B. Solvent compositions of the mobile phase remained as such (70%A:30%B) for a period of two minutes before being changed to 100%B. At time = 34 minutes, the solvent composition was again changed to 80%A:20%B for column re-equilibration. The flow rate of the mobile phase was 1.5mL/minute. The chart speed of the integrator was 0.5cm/minute. The results of these analyses are shown in Table 20.

In vivo analysis of the DOTA-BBN analogs in CF1 mice demonstrates that ^{149}Pm-DOTA-5-Ava-BBN[7-14]NH_2 and ^{149}Pm-DOTA-8-Aoc-BBN[7-14]NH_2 target GRP receptor expression *in vivo* based on high uptake and accumulation of these compounds in the normal pancreas, which contain high levels of the GRP receptor [Tables 15 and 16].

In vivo analysis of the DOTA-BBN analogs in CF1 mice demonstrates that ^{177}Lu-DOTA-5-Ava-BBN[7- 1 4]NH_2, ^{177}Lu-DOTA-8-Aoc-BBN[7-14]NH_2, and ^{177}Lu -DOTA-11-Aun-BBN[7-14]NH_2 all target GRP receptor expression *in vivo* based on high uptake and accumulation of these compounds in the normal pancreas [Tables 17 and 18]

The biodistribution studies of ^{177}Lu-DOTA-8-Aoc-BBN[7-14]NH_2 were determined in SCID mice bearing human prostate cancer, PC-3 tumors. The mice were injected with 4μCi of the complex in 100μL of isotonic saline via the tail vein. The mice were euthanized by cervical dislocation. Tissues and organs were excised from the animals following at 1 hour, 4 hours, and 24 hours post-injection (p.i.). Subsequently, the tissues and organs were weighed and counted in a NaI well counter and the percent injected dose (%ID) and %ID/g of each organ or tissue calculated. The %ID in whole blood was estimated assuming a whole-blood volume of 6.5% the total body weight.

Prolonged PC-3 human prostate tumor uptake was demonstrated for ^{177}Lu-DOTA-8-Aoc-BBN[7-14] using a xenograft mouse model of human prostate cancer [Tables 19 and 20].

CONCLUSION: This pre-clinical evaluation of ^{177}Lu-DOTA-8-Aoc-BBN[7-14]NH_2 and ^{149}Pm-DOTA-8-Aoc-BBN[7-14]NH_2 suggests the potential for peptide conjugates of this type to be used as site-directed, therapeutic radiopharmaceuticals.

Table B. Biodistribution of ^{99m}Tc-BBN-122 in normal CF-1 mice at 0.5, 1, 4 and 24 hours post-IV injection. Results expressed as % ID/organ

Organ[c]	%Injected Dose/Organ[a]			
	30 min	1 hr	4 hr	24 hr
Blood[d]	3.52 ± 2.16	1.08 ± 0.34	0.59 ± 0.24	0.12 ± 0.01
Liver	4.53 ± 0.93	4.77 ± 1.40	1.49 ± 0.32	0.32 ± 0.06
Stomach	2.31 ± 0.45	1.61 ± 0.81	1.75 ± 0.20	0.30 ± 0.06
Lg. Intestine[b]	2.84 ± 0.32	24.17 ± 7.91	23.85 ± 7.02	0.61 ± 0.14

Sm. Intestine[b]	43.87 ± 1.51	23.91 ± 9.08	5.87 ± 7.09	0.42 ± 0.06
Kidneys[b]	1.49 ± 0.19	1.15 ± 0.10	0.55 ± 0.06	0.20 ± 0.01
Urine[b]	26.78 ± 1.05	33.79 ± 1.76	~35	~35
Muscle	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Pancreas	5.30 ± 0.63	3.20 ± 0.83	1.21 ± 0.13	0.42 ± 0.17

a. Each value in the table represents the mean and SD from 5 animals in each group

b. At 4 and 24 hours, feces containing ^{99m}Tc had been excreted from each animal and the % ID in the urine was estimated to be approximately 60% of the ID.

c. All other organs excised (incl. Brain, heart, lung and spleen) shown < 0.10% at t ≥ 1 hr.

d. % ID in the blood estimated assuming the whole blood volume is 6:5% of the body weight.

Table C. Biodistribution of ^{99m}Tc-BBN-122 in normal CF-1 mice at 0.5, 1, 4 and 24 hours post I.V. injection. Results expressed as % ID/gm.

	%Injected Dose/gma			
Organ	30 min	1 hr	4 hr	24 hr
Blood[b]	2.00 ± 1.28	0.63 ± 0.19	0.34 ± 0.11	0.08 ± 0.00
Liver	2.70 ± 0.41	3.14 ± 0.81	0.96 ± 0.20	0.22 ± 0.05
Kidneys	3.99 ± 0.76	3.10 ± 0.31	1.58 ± 0.15	0.64 ± 0.07
Muscle	0.23 ± 0.08	0.13 ± 0.02	0.05 ± 0.01	0.01 ± 0.01

Pancreas	16.89 ± 0.95	12.63 ± 1.87	5.05 ± 0.42	1.79 ± 0.71
P/B1 and P/M Update Ratios				
Pancreas/Blood	8.42	19.76	14.78	20.99
Pancreas/Muscle	73.16	93.42	92.25	142.76

a. Each value in the table represents the mean and SD from 5 animals in each group.

b. % ID in the blood estimated assuming the whole blood volume is 6:5% of the body weight.

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Table D. Biodistribution of ^{99m}Tc-BBN-122 in PC-3 tumor bearing SCID mice at 1, 4 and 24 hr post-I.V. injection. Results expressed as % ID/organ.

Tumor Line: PC-3	% ID per Organ[a]		
Organ[c]	1 hr	4 hr	24 hr
Blood[b]	1.16 ± 0.27	0.47 ± 0.06	0.26 ± 0.05
Liver	1.74 ± 0.64	0.72 ± 0.10	0.29 ± 0.05
Stomach	0.43 ± 0.18	0.29 ± 0.22	0.08 ± 0.02
Lg. Intestine	9.18 ± 19.42	42.55 ± 8.74	0.64 ± 0.17
Sm. Intestine	46.55 ± 16.16	2.13 ± 0.76	0.31 ± 0.04
Kidneys	1.16 ± 0.20	0.60 ± 0.06	0.16 ± 0.01
Urine[d]	32.05 ± 12.78	~35	~35
Muscle	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pancreas	1.69 ± 0.61	1.05 ± 0.13	0.34 ± 0.08
Tumor	1.00 ± 0.78	0.49 ± 0.08	0.49 ± 0.25

a. Each value in the table represents the mean and SD from 5 animals in each group.

b. At 4 and 24 hr, feces containing ^{99m}Tc had been excreted from each animal and the % ID in the urine was estimated to be approximately 60% of the ID.

c. All other organs excised (incl. brain, heart, lung and spleen) showed $< 0.10\%$ at $t \geq 1$ hr.

d. % ID in the blood estimated assuming the whole blood volume is 6:5% of the body weight.

Table E. Biodistribution of ^{99m}Tc-BBN-122 in PC-3 tumor bearing SCID mice at 1, 4 and 24 hr post-I.V. injection. Results expressed as % ID/Gm.

Tumor Line: PC-3	% ID per gm[a]		
Organ	1 hr	4 hr	24 hr
Blood[b]	0.97 ± 0.26	0.31 ± 0.03	0.18 ± 0.04
Liver	2.07 ± 0.88	0.64 ± 0.05	0.26 ± 0.04
Kidneys	4.80 ± 1.33	2.23 ± 0.35	0.60 ± 0.04
Muscle	0.18 ± 0.12	0.06 ± 0.03	0.05 ± 0.04
Pancreas	10.34 ± 3.38	5.08 ± 1.12	1.47 ± 0.23
Tumor	2.07 ± 0.50	1.75 ± 0.61	1.28 ± 0.22
T/Bl, T/M, P/Bl and P/M Uptake Ratios			
Tumor/Blood	2.13	5.52	6.79
Tumor/Muscle	11.44	25.38	21.62
Pancreas/Blood	10.64	15.96	7.81
Pancreas/Muscle	57.14	73.40	24.87

a. Each value in the table represents the mean and SD from 5 animals in each group.
b. % ID in the blood estimated assuming the whole blood volume is 6.5% of the body weight.

The invention has been described in an illustrative manner, and it is to be understood that the terminology that has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

Throughout this application, various publications are referenced by citation and number. Full citations for the publication are listed below. The disclosure of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Table 1

Binding Affinity of Rh-BBN-37 for GRP Receptors Expressed on Neoplasms

Type of Cancer	Cell Line	IC_{50} (Mean Value)
Pancreatic CA	CF PAC1	3.2×10^{-9}
Prostate CA	PC-3	7.0×10^{-9}

Table 2
(%Dose)

Complex Organ (%Dose)	^{105}Rh-Peptide22 30 min n=9	^{105}Rh-Peptide22 1 hr n=9	^{105}Rh-Peptide22 2 hr n=9
Brain	0.08 ± 0.02	0.04 ± 0.01	0.06 ± 0.09
Blood	4.48 ± 1.24	1.86 ± 0.38	0.99 ± 0.24
Heart	0.13 ± 0.03	0.08 ± 0.03	0.04 ± 0.04
Lung	0.25 ± 0.08	0.20 ± 0.09	0.15 ± 0.09
Liver	7.97 ± 2.85	8.51 ± 2.33	8.57 ± 2.04
Spleen	0.07 ± 0.03	0.09 ± 0.08	0.05 ± 0.01
Stomach	1.11 ± 0.76	0.59 ± 0.21	0.30 ± 0.16
Large Intestine	0.73 ± 0.16	3.21 ± 3.38	8.91 ± 3.79
Small Intestine	6.29 ± 1.87	6.98 ± 1.87	3.48 ± 1.78
Kidneys	4.25 ± 1.33	3.25 ± 0.60	2.44 ± 0.64
Bladder	44.66 ± 7.29	62.88 ± 3.84	68.41 ± 6.63
Muscle	0.06 ± 0.03	0.03 ± 0.03	0.01 ± 0.01
Pancreas	0.95 ± 0.46	1.15 ± 0.49	1.01 ± 0.14
Carcass	32.90 ± 6.61	12.62 ± 4.77	6.37 ± 1.17

Table 2 (continued)
(%Dose/Gm)

Complex Organ (%D/GM)	^{105}Rh-Peptide22 30 min n=9	^{105}Rh-Peptide22 1 hr n=9	^{105}Rh-Peptide22 2 hr n=9
Brain	0.21 ± 0.07	0.14 ± 0.08	0.16 ± 0.28
Blood	2.22 ± 0.40	1.02 ± 0.22	0.51 ± 0.11
Heart	0.92 ± 0.25	0.64 ± 0.20	0.38 ± 0.33
Lung	1.44 ± 0.33	1.24 ± 0.54	0.92 ± 0.69
Liver	4.33 ± 1.52	5.18 ± 1.52	5.17 ± 1.12
Spleen	0.86 ± 0.38	1.10 ± 0.65	0.84 ± 0.53
Stomach	2.46 ± 1.65	1.53 ± 0.74	0.71 ± 0.33
Large Intestine	0.78 ± 0.19	4.42 ± 4.62	10.10 ± 4.58
Small Intestine	4.73 ± 1.47	5.84 ± 1.81	2.86 ± 1.47
Kidneys	7.57 ± 1.49	6.70 ± 0.75	4.60 ± 0.83
Muscle	0.53 ± 0.32	0.61 ± 0.97	0.24 ± 0.24
Pancreas	3.12 ± 0.99	4.31 ± 1.98	3.88 ± 1.25

Table 33
(%Dose)

Complex / Organ (%Dose)	^{105}Rh-Pept37 30 min n=5	^{105}Rh-Pept37 1 hr n=9	^{105}Rh-Pept37 2 hr n=7
Brain	0.03 ± 0.01	0.07 ± 0.11	0.03 ± 0.03
Blood	3.09 ± 0.54	1.46 ± 0.62	0.66 ± 0.26
Heart	0.12 ± 0.03	0.05 ± 0.03	0.04 ± 0.02
Lung	0.26 ± 0.09	0.12 ± 0.07	0.08 ± 0.11
Liver	13.04 ± 1.93	13.00 ± 3.59	10.12 ± 1.86
Spleen	0.21 ± 0.13	0.16 ± 0.08	0.10 ± 0.04
Stomach	0.60 ± 0.34	0.65 ± 0.52	0.83 ± 0.96
Large Intestine	2.05 ± 0.69	2.96 ± 1.67	8.07 ± 2.25
Small Intestine	8.44 ± 1.89	11.38 ± 3.02	5.04 ± 2.27
Kidneys	7.82 ± 2.52	6.04 ± 1.68	4.57 ± 1.29
Bladder	39.65 ± 7.21	51.82 ± 7.53	62.32 ± 5.78
Muscle	0.06 ± 0.03	0.02 ± 0.01	0.02 ± 0.02
Pancreas	2.73 ± 1.14	3.63 ± 1.22	2.25 ± 1.02
Carcass	24.35 ± 7.69	9.81 ± 2.91	6.37 ± 1.73

Table 3 (continued)
(%Dose/Gm)

Complex Organ (%D/GM)	^{105}Rh-Pept37 30 min n=5	^{105}Rh-Pept37 1 hr n=9	^{105}Rh-Pept37 2 hr n=7
Brain	0.10 ± 0.05	0.26 ± 0.41	0.10 ± 0.09
Blood	1.60 ± 0.30	0.72 ± 0.31	0.34 ± 0.15
Heart	0.92 ± 0.26	0.38 ± 0.21	0.28 ± 0.17
Lung	1.52 ± 0.48	0.76 ± 0.47	0.46 ± 0.50
Liver	7.31 ± 1.15	7.65 ± 1.29	6.30 ± 1.73
Spleen	2.18 ± 1.17	1.59 ± 0.71	1.05 ± 0.44
Stomach	1.53 ± 0.67	1.63 ± 1.17	2.18 ± 2.35
Large Intestine	2.46 ± 0.70	3.80 ± 2.42	11.84 ± 4.39
Small Intestine	5.69 ± 1.26	7.85 ± 1.87	3.81 ± 2.01
Kidneys	14.28 ± 2.84	11.21 ± 3.68	8.39 ± 2.36
Muscle	0.73 ± 0.39	0.20 ± 0.14	0.39 ± 0.38
Pancreas	14.02 ± 3.23	15.54 ± 6.21	9.91 ± 5.35

Table 4

ES-MS and HPLC data of DOTA-BBN[7-14]NH_2 and In-DOTA-BBN[7-14]NH_2 analogues.

BBN Analogue	ES-MS			HPLC t_r (min)[a]
	Mol. Formula	Calculated	Observed	
0	$C_{59}H_{91}N_{17}O_{16}S$	1326.5	1326.6	13.2
3	$C_{62}H_{96}N_{18}O_{17}S$	1397.6	1397.4	13.4
5	$C_{64}H_{100}N_{18}O_{17}S$	1425.7	1425.8	14.0
8	$C_{67}H_{106}N_{18}O_{17}S$	1467.8	1467.8	19.1
11	$C_{70}H_{112}N_{18}O_{17}S$	1509.8	1509.8	17.1[b]
In-0	$C_{59}H_{88}N_{17}O_{16}SIn$	1438.3	1438.2	12.9
In-3	$C_{62}H_{93}N_{18}O_{17}SIn$	1509.4	1509.6	12.7
In-5	$C_{64}H_{97}N_{18}O_{17}SIn$	1536.5	1537.7	13.6
In-8	$C_{67}H_{103}N_{18}O_{17}SIn$	1579.6	1579.7	19.0
In-11	$C_{70}H_{109}N_{18}O_{17}SIn$	1621.6	1621.7	16.8[b]

Table 5

IC_{50} (nM) values (n = 3 or 4 separate experiments performed in duplicate) of In-DOTA-BBN[7-14]NH_2 analogues vs. ^{125}I-Tyr^4-BBN in human prostate PC-3 cells and human breast carcinoma T47D cells.

BBN Analogue	PC-3 IC_{50} (nM)	T47D IC_{50} (nM)
0	110.6 ± 32.3	322 ± 54.5
β-Ala	2.1 ± 0.3	4.7 ± 0.7
5-Ava	1.7 ± 0.4	2.3 ± 1.01
8-Aoc	0.6 ± 0.1	1.3 ± 0.21
11-Aun	64.0 ± 11.2	516 ± 32.2

Table 6

^{111}In-DOTA-SPACER-BBN[7-14]NH_2 biodistribution (Avg %ID/gm, n = 5) in CF1 normal mice after 1 hour post-injection.

Spacer Tissue	0	β-Ala	5-Ava	8-Aoc	11-Aun
Blood	0.10 ± 0.03	0.11 ± 0.06	0.20 ± 0.07	0.32 ± 0.09	0.34 ± 0.08
Heart	0.05 ± 0.02	0.06 ± 0.04	0.10 ± 0.04	0.05 ± 0.02	0.13 ± 0.04
Lung	0.13 ± 0.03	0.11 ± 0.08	0.20 ± 0.06	0.31 ± 0.07	0.26 ± 0.05
Liver	0.09 ± 0.01	0.11 ± 0.02	0.16 ± 0.02	0.65 ± 0.07	1.22 ± 0.25
Spleen	0.08 ± 0.02	0.37 ± 0.06	0.87 ± 0.28	1.51 ± 0.41	1.15 ± 0.38
Stomach	0.06 ± 0.03	0.30 ± 0.07	0.71 ± 0.24	1.02 ± 0.26	1.05 ± 0.25
L. Intestine	0.09 ± 0.03	1.10 ± 0.78	3.07 ± 0.86	2.66 ± 1.07	4.34 ± 1.34
S. Intestine	0.44 ± 0.64	1.01 ± 0.37	3.49 ± 0.87	4.43 ± 0.90	11.12 ± 2.07
Kidney	1.24 ± 0.14	1.40 ± 0.27	1.84 ± 0.44	2.37 ± 0.31	2.06 ± 0.31
Muscle	0.03 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.12 ± 0.05	0.09 ± 0.03
Pancreas	0.20 ± 0.04	4.92 ± 0.37	15.78 ± 2.54	26.97 ± 3.97	26.00 ± 3.46

Table 7

^{111}In-DOTA-SPACER-BBN[7-14]NH_2 biodistribution (Avg %ID, n = 5) in CF1 normal mice after 1 hour post-injection.

Spacer Tissue	0	β-Ala	5-Ava	8-Aoc	11-Aun
Blood	0.22 ± 0.07	0.23 ± 0.10	0.45 ± 0.14	0.66 ± 0.13	0.79 ± 0.20
Heart	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01
Lung	0.03 ± 0.00	0.03 ± 0.02	0.04 ± 0.01	0.07 ± 0.02	0.08 ± 0.01
Liver	0.17 ± 0.02	0.17 ± 0.03	0.26 ± 0.03	1.02 ± 0.08	2.44 ± 0.50
Spleen	0.01 ± 0.00	0.05 ± 0.00	0.11 ± 0.04	0.17 ± 0.04	0.19 ± 0.04
Stomach	0.03 ± 0.01	0.13 ± 0.02	0.37 ± 0.16	0.50 ± 0.06	0.53 ± 0.11
L. Intestine	0.10 ± 0.02	0.90 ± 0.57	2.74 ± 0.80	3.02 ± 0.33	5.54 ± 2.42
S. Intestine	0.25 ± 0.04	1.57 ± 0.65	0.11 ± 0.04	6.58 ± 1.10	17.84 ± 1.40
Kidney	0.57 ± 0.02	0.62 ± 0.10	0.75 ± 0.14	1.04 ± 0.12	1.07 ± 0.17
Urine	96.95 ± 0.37	92.41 ± 0.90	81.29 ± 1.32	71.61 ± 1.82	53.26 ± 0.90
Muscle	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Pancreas	0.07 ± 0.01	1.84 ± 0.35	5.57 ± 0.99	10.81 ± 0.78	11.56 ± 1.14
Carcass	1.78 ± 0.37	2.23 ± 0.36	3.15 ± 0.70	5.01 ± 0.47	7.35 ± 1.57

Table 8

^{111}In-DOTA-BBN[7-14]NH_2 analogues biodistribution (Avg %ID/gm, n = 5) in CF1 normal mice after 1 hour post-injection.

Analogue Tissue	8-Aoc	8-Aoc Blocking
Blood	0.32 ± 0.09	0.49 ± 0.15
Heart	0.05 ± 0.02	0.16 ± 0.06
Lung	0.31 ± 0.07	0.74 ± 0.17
Liver	0.65 ± 0.07	0.54 ± 0.13
Spleen	1.51 ± 0.41	0.15 ± 0.16
Stomach	1.02 ± 0.26	0.32 ± 0.34
L. Intestine	2.66 ± 1.07	0.16 ± 0.06
S. Intestine	4.43 ± 0.90	0.95 ± 0.18
Kidney	2.37 ± 0.31	2.19 ± 0.47
Muscle	0.12 ± 0.05	0.11 ± 0.07
Pancreas	26.97 ± 3.97	0.43 ± 0.10

Table 9

^{111}In-DOTA-BBN[7-14]NH_2 analogues biodistribution (Avg %ID, n = 5) in CF1 normal mice after 1 hour post-injection.

Analogue Tissue	8-Aoc	8-Aoc Blocking
Blood	0.66 ± 0.13	0.98 ± 0.23
Heart	0.01 ± 0.00	0.03 ± 0.01
Lung	0.07 ± 0.02	0.17 ± 0.05
Liver	1.02 ± 0.08	0.87 ± 0.10
Spleen	0.17 ± 0.04	0.02 ± 0.03
Stomach	0.50 ± 0.06	0.16 ± 0.12
L. Intestine	3.02 ± 0.33	0.15 ± 0.05
S. Intestine	6.58 ± 1.10	1.65 ± 0.19
Kidney	1.04 ± 0.12	0.92 ± 0.13
Urine	71.61 ± 1.82	88.19 ± 1.79
Muscle	0.02 ± 0.01	0.02 ± 0.01
Pancreas	10.81 ± 0.78	0.19 ± 0.03
Carcass	5.01 ± 0.47	7.42 ± 1.35

Table 10

^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 biodistribution (Avg %ID/gm, n = 5) in PC-3 tumor bearing mice.

Time Tissue	15 min	30 min	1 hr	4 hrs	24 hrs	48 hrs	72 hrs
Blood	5.585 ± 2.43	1.46 ± 0.44	0.60 ± 0.39	0.27 ± 0.02	0.10 ± 0.03	0.07 ± 0.03	0.01 ± 0.02
Heart	2.20 ± 1.05	0.62 ± 0.33	0.25 ± 0.18	0.13 ± 0.06	0.05 ± 0.09	0.05 ± 0.05	0.01 ± 0.01
Lung	3.35 ± 1.22	0.94 ± 0.28	0.50 ± 0.39	0.25 ± 0.08	0.09 ± 0.07	0.06 ± 0.02	0.02 ± 0.02
Liver	2.03 ± 0.85	0.70 ± 0.21	1.34 ± 0.25	1.44 ± 0.57	0.37 ± 0.12	0.13 ± 0.04	0.07 ± 0.02
Spleen	2.21 ± 0.80	0.83 ± 0.26	1.39 ± 1.17	1.59 ± 0.27	0.46 ± 0.20	0.22 ± 0.22	0.08 ± 0.09
Stomach	3.30 ± 1.99	1.82 ± 0.44	1.99 ± 0.24	0.96 ± 0.57	0.30 ± 0.05	0.12 ± 0.03	0.05 ± 0.02
L. Intestine	8.58 ± 3.04	4.33 ± 0.44	4.29 ± 2.55	10.27 ± 2.70	2.35 ± 0.43	0.81 ± 0.20	0.45 ± 0.04
S. Intestine	7.82 ± 2.26	5.16 ± 1.06	6.80 ± 1.81	2.24 ± 0.35	0.89 ± 0.16	0.25 ± 0.06	0.12 ± 0.02
Kidney	29.03 ± 14.40	8.70 ± 2.80	5.66 ± 1.33	3.18 ± 0.43	1.18 ± 0.14	0.48 ± 0.09	0.20 ± 0.02
Muscle	1.30 ± 0.60	0.32 ± 0.12	0.08 ± 0.07	0.04 ± 0.02	0.05 ± 0.05	0.02 ± 0.04	0.01 ± 0.02
Pancreas	54.33 ± 9.70	27.87 ± 3.44	18.80 ± 10.97	16.55 ± 4.43	6.78 ± 1.15	0.77 ± 0.44	0.23 ± 0.08
Tumor	7.59 ± 2.11	4.58 ± 0.53	3.63 ± 1.11	1.78 ± 1.09	1.56 ± 0.45	0.68 ± 0.24	0.34 ± 0.10

^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 biodistribution (Avg %ID, n = 5) in PC-3 tumor bearing mice.

Time Tissue	15 min	30 min	1 hr	4 hrs	24 hrs	48 hrs	72 hrs
Blood	7.92± 2.03	2.47 ± 0.74	0.92 ± 0.58	0.40 ± 0.11	0.15 ± 0.04	0.12 ± 0.05	0.02 ± 0.03
Heart	0.20 ± 0.07	0.06 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Lung	0.62 ± 0.25	0.20 ± 0.06	0.09 ± 0.07	0.05 ± 0.02	0.02 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
Liver	1.85 ± 0.41	0.85 ± 0.21	1.41 ± 0.32	1.57 ± 0.72	0.40 ± 0.17	0.15 ± 0.04	0.08 ± 0.01
Spleen	0.10 ± 0.02	0.08 ± 0.03	0.09 ± 0.07	0.10 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01
Stomach	0.98 ± 0.18	0.65 ± 0.04	0.52 ± 0.11	0.34 ± 0.23	0.09 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
L. Intestine	5.84 ± 0.90	4.53 ± 0.45	2.18 ± 0.86	6.04 ± 2.05	1.46 ± 0.42	0.73 ± 0.21	0.41 ± 0.05
S. Intestine	6.98 ± 0.35	6.24 ± 0.46	7.45 ± 1.62	2.43 ± 0.56	0.98 ± 0.26	0.32 ± 0.07	0.15 ± 0.02
Kidney	7.18 ± 3.16	2.45 ± 0.78	1.81 ± 0.37	0.97 ± 0.08	0.36 ± 0.08	0.16 ± 0.04	0.06 ± 0.01
Urine	27.91 ±10.33	62.61 ± 5.02	68.56 ± 6.96	81.83 ± 3.82	87.15 ± 4.31	91.75 ± 4.13	92.53 ± 1.09
Muscle	0.16 ± 0.06	0.05 ± 0.02	0.01 ± 0.01	0.0 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Feces	-	-	-	-	6.10 ± 2.60	5.89 ± 3.81	6.23 ± 1.00
Pancreas	10.26 ± 1.44	7.37 ± 1.20	3.49 ± 2.15	3.28 ± 0.80	1.19 ± 0.49	0.17 ± 0.09	0.06 ± 0.02
Carcass	33.06 ± 5.51	12.31 ± 3.27	13.55 ± 6.05	2.92 ± 0.64	1.69 ± 0.56	0.57 ± 0.02	0.38 ± 0.06
Tumor	1.92 ± 1.22	0.99 ± 0.62	0.36 ± 0.32	0.18 ± 0.17	0.25 ± 0.13	0.08 ± 0.05	0.03 ± 0.01

Table 11

Table 12

^{90}Y-DOTA-8-Aoc-BBN[7-14]NH_2 biodistribution (Avg %ID/gm) in PC-3 tumor bearing mice.

Time Tissue	1 hr (n = 9)	4 hrs (n = 9)	24 hrs (n = 6)	48 hrs (n = 6)	72 hrs (n = 6)
Blood	0.34 ± 0.12	0.05 ± 0.07	0.07 ± 0.08	0.06 ± 0.08	0.06 ± 0.09
Heart	0.10 ± 0.11	0.10 ± 0.14	0.00 ± 0.00	0.14 ± 0.19	0.36 ± 0.45
Lung	0.22 ± 0.12	0.07 ± 0.07	0.01 ± 0.02	0.03 ± 0.03	0.02 ± 0.03
Liver	0.39 ± 0.29	0.18 ± 0.12	0.08 ± 0.03	0.03 ± 0.03	0.08 ± 0.11
Spleen	1.09 ± 0.67	0.35 ± 0.42	0.11 ± 0.13	0.09 ± 0.24	0.28 ± 0.26
Stomach	1.34 ± 0.64	0.55 ± 0.16	0.09 ± 0.07	0.04 ± 0.07	0.07 ± 0.03
L. Intestine	3.35 ± 1.12	5.17 ± 1.85	1.27 ± 0.92	0.77 ± 0.15	0.47 ± 0.24
S. Intestine	3.64 ± 0.82	1.66 ± 0.91	0.35 ± 0.16	0.13 ± 0.04	0.08 ± 0.05
Kidney	3.77 ± 1.41	1.68 ± 0.76	0.51 ± 0.25	0.29 ± 0.12	0.43 ± 0.29
Muscle	0.15 ± 0.19	0.07 ± 0.13	0.02 ± 0.03	0.07 ± 0.13	0.08 ± 0.19
Pancreas	24.73 ± 4.97	14.02 ± 4.89	1.80 ± 0.57	0.59 ± 0.14	0.27 ± 0.17
Tumor	2.95 ± 0.99	1.98 ± 0.66	1.08 ± 0.37	0.58 ± 0.30	0.46 ± 0.48

Table 13

^{90}Y-DOTA-8-Aoc-BBN[7-14]NH_2 biodistribution (Avg %ID) in PC-3 tumor bearing mice.

Time Tissue	1 hr (n = 9)	4 hrs (n = 9)	24 hrs (n = 6)	48 hrs (n = 6)	72 hrs (n = 6)
Blood	0.56 ± 0.20	0.09 ± 0.13	0.12 ± 0.14	0.10 ± 0.14	0.11 ± 0.15
Heart	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.02	0.04 ± 0.04
Lung	0.06 ± 0.03	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.01
Liver	0.44 ± 0.34	0.19 ± 0.13	0.10 ± 0.03	0.04 ± 0.04	0.08 ± 0.11
Spleen	0.07 ± 0.04	0.02 ± 0.03	0.01 ± 0.01	0.01 ± 0.02	0.02 ± 0.01
Stomach	0.41 ± 0.12	0.18 ± 0.06	0.04 ± 0.04	0.01 ± 0.02	0.04 ± 0.02
L. Intestine	2.44 ± 0.66	3.33 ± 0.71	1.11 ± 0.75	0.47 ± 0.12	0.35 ± 0.20
S. Intestine	4.65 ± 0.98	2.06 ± 1.27	0.51 ± 0.20	0.16 ± 0.06	0.11 ± 0.07
Kidney	1.22 ± 0.46	0.54 ± 0.25	0.17 ± 0.09	0.10 ± 0.04	0.13 ± 0.07
Urine	57.73 ± 14.52	67.02 ± 16.74	67.62 ± 17.26	76.74 ± 21.06	82.97 ± 25.39
Muscle	0.02 ± 0.02	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.02	0.01 ± 0.02
Feces	-	-	10.71 ± 8.29	6.78 ± 3.88	13.89 ± 4.48
Pancreas	5.70 ± 1.60	3.42 ± 0.82	0.44 ± 0.08	0.15 ± 0.04	0.08 ± 0.05
Carcass	0.62 ± 0.44	0.14 ± 0.11	0.04 ± 0.04	0.11 ± 0.17	0.10 ± 0.11
Tumor	0.40 ± 0.22	0.34 ± 0.22	0.16 ± 0.09	0.11 ± 0.07	0.06 ± 0.06

Table 14

In Vivo Biodistribution Analyses (%ID/g (SD), n=5) of ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 in Tumor-Bearing Mice Models (MDA-MB-231).

Tissue/Organ	1 hour	4 hours	24 hours
Blood	0.35 ± 0.08	0.08 ± 0.10	0.02 ± 0.03
Heart	0.15 ± 0.11	0.03 ± 0.05	0.08 ± 0.06
Lung	0.31 ± 0.09	0.06 ± 0.06	0.05 ± 0.05
Liver	0.31 ± 0.04	0.15 ± 0.09	0.07 ± 0.02
Spleen	0.57 ± 0.10	0.48 ± 0.25	0.21 ± 0.07
Stomach	1.49 ± 0.68	0.27 ± 0.08	0.33 ± 0.10
L. Intestine	5.14 ± 0.42	5.58 ± 1.26	2.76 ± 0.49
S. Intestine	5.15 ± 0.19	1.52 ± 0.19	0.90 ± 0.14
Kidney	3.29 ± 0.56	1.76 ± 0.15	0.98 ± 0.28
Pancreas	23.4 ± 4.99	17.9 ± 5.00	5.06 ± 0.77
Muscle	0.08 ± 0.05	0.06 ± 0.13	0.03 ± 0.05
Tumor 1	0.91 ± 0.16	0.36 ± 0.13	0.22 ± 0.07
Tumor 2	0.74 ± 0.27	0.40 ± 0.23	0.24 ± 0.15
Urine (%ID)	72.1±3.55	84.3 ± 2.09	83.8 ± 1.41

Table 15

^{149}Pm-DOTA-SPACER-BBN[7-14]NH_2 biodistribution (Avg %ID/gm, n = 5) in CF1 normal mice after 1 hour post-injection.

Spacer Tissue	0	β-Ala	5-Ava	8-Aoc
Blood	0.00 ± 0.00	0.21 ± 0.22	0.27 ± 0.06	0.12 ± 0.13
Heart	0.00 ± 0.00	0.17 ± 0.24	0.42 ± 0.59	0.03 ± 0.06
Lung	0.00 ± 0.00	0.34 ± 0.30	0.78 ± 1.08	0.09 ± 0.14
Liver	0.12 ± 0.10	0.15 ± 0.05	0.23 ± 0.13	0.19 ± 0.12
Spleen	0.00 ± 0.00	0.16 ± 0.31	2.37 ± 1.36	1.61 ± 0.36
Stomach	0.04 ± 0.08	0.19 ± 0.11	1.90 ± 1.60	1.16 ± 0.59
L. Intestine	0.01 ± 0.03	0.42 ± 0.08	3.53 ± 1.10	4.14 ± 2.14
S. Intestine	0.27 ± 0.17	0.63 ± 0.20	5.15 ± 1.20	12.56 ± 16.70
Kidney	1.04 ± 0.90	2.05 ± 1.63	2.81 ± 0.66	3.74 ± 1.02
Muscle	0.00 ± 0.00	0.04 ± 0.10	0.24 ± 0.25	0.09 ± 0.21
Pancreas	0.00 ± 0.00	2.40 ± 1.33	22.1 ± 5.40	28.29 ± 13.26

Table 16

^{149}Pm-DOTA-SPACER-BBN[7-14]NH_2 biodistribution (Avg %ID, n = 5) in CF1 normal mice after 1 hour post-injection.

Spacer Tissue	0	β-Ala	5-Ava	8-Aoc
Blood	0.00 ± 0.00	0.30 ± 0.32	0.47 ± 0.11	0.23 ± 0.26
Heart	0.00 ± 0.00	0.02 ± 0.02	0.06 ± 0.09	0.00 ± 0.01
Lung	0.00 ± 0.00	0.06 ± 0.04	0.17 ± 0.21	0.02 ± 0.04
Liver	0.16 ± 0.15	0.23 ± 0.07	0.37 ± 0.20	0.35 ± 0.20
Spleen	0.00 ± 0.00	0.02 ± 0.04	0.27 ± 0.13	0.24 ± 0.06
Stomach	0.02 ± 0.05	0.10 ± 0.03	0.77 ± 0.74	0.66 ± 0.35
L. Intestine	0.01 ± 0.02	0.31 ± 0.06	3.18 ± 1.18	4.43 ± 2.37
S. Intestine	0.38 ± 0.25	0.95 ± 0.19	7.70 ± 0.66	7.84 ± 2.15
Kidney	0.34 ± 0.28	0.61 ± 0.41	1.11 ± 0.29	1.55 ± 0.47
Urine	97.10 ± 2.91	95.54 ± 1.15	75.82 ± 2.02	67.20 ± 5.53
Muscle	0.00 ± 0.00	0.00 ± 0.01	0.03 ± 0.04	0.01 ± 0.02
Pancreas	0.07 ± 0.01	0.46 ± 0.23	4.25 ± 0.43	7.34 ± 3.51
Carcass	1.98 ± 2.27	1.64 ± 0.38	6.16 ± 0.75	10.30 ± 1.84

Table 17

^{177}Lu-DOTA-SPACER-BBN[7-14]NH_2 biodistribution (Avg %ID/gm, n = 5) in CF1 normal mice after 1 hour post-injection.

Spacer Tissue	0	β-Ala	5-Ava	8-Aoc	11-Aun
Blood	0.58 ± 0.96	0.16 ± 0.17	0.22 ± 0.19	0.14 ± 0.10	0.78 ± 1.10
Heart	0.04 ± 0.09	0.43 ± 0.70	0.34 ± 0.35	0.19 ± 0.36	1.56 ± 2.40
Lung	0.19 ± 0.26	0.23 ± 0.33	0.47 ± 0.84	0.20 ± 0.21	0.73 ± 0.81
Liver	0.09 ± 0.06	0.15 ± 0.06	0.09 ± 0.04	0.23 ± 0.05	1.65 ± 0.29
Spleen	0.04 ± 0.09	0.31 ± 0.31	1.26 ± 0.69	1.23 ± 0.59	1.78 ± 1.87
Stomach	0.10 ± 0.21	0.34 ± 0.18	1.48 ± 2.25	1.41 ± 0.44	1.82 ± 1.12
L. Intestine	0.07 ± 0.09	0.45 ± 0.19	3.78 ± 1.23	6.17 ± 0.79	6.31 ± 0.86
S. Intestine	0.75 ± 0.60	0.49 ± 0.10	2.55 ± 1.31	6.47 ± 1.24	12.58 ± 1.73
Kidney	1.21 ± 0.31	1.88 ± 0.37	2.03 ± 1.02	4.97 ± 0.71	4.97 ± 0.61
Muscle	0.09 ± 0.15	0.94 ± 1.54	0.67 ± 0.90	0.17 ± 0.39	0.75 ± 1.12
Pancreas	0.18 ± 0.28	1.44 ± 0.26	16.41 ± 1.38	30.83 ± 1.89	35.48 ± 2.39

Table 18

^{177}Lu-DOTA-SPACER-BBN[7-14]NH_2 biodistribution (Avg %ID, n = 5) in CF1 normal mice after 1 hour post-injection.

Spacer Tissue	0	β-Ala	5-Ava	8-Aoc	11-Aun
Blood	0.39 ± 0.34	0.24 ± 0.25	0.35 ± 0.31	0.20 ± 0.15	0.47 ± 0.54
Heart	0.01 ± 0.02	0.05 ± 0.08	0.04 ± 0.05	0.02 ± 0.04	0.19 ± 0.29
Lung	0.04 ± 0.06	0.04 ± 0.06	0.08 ± 0.15	0.03 ± 0.04	0.17 ± 0.22
Liver	0.19 ± 0.10	0.21 ± 0.09	0.14 ± 0.06	0.31 ± 0.05	2.26 ± 0.46
Spleen	0.01 ± 0.01	0.05 ± 0.04	0.18 ± 0.12	0.16 ± 0.05	0.24 ± 0.24
Stomach	0.05 ± 0.11	0.13 ± 0.09	0.73 ± 1.33	0.51 ± 0.15	0.64 ± 0.35
L. Intestine	0.09 ± 0.12	0.36 ± 0.17	3.52 ± 1.37	4.63 ± 0.57	5.03 ± 0.46
S. Intestine	1.27 ± 1.03	0.64 ± 0.20	3.80 ± 1.87	9.55 ± 2.37	17.10 ± 3.60
Kidney	0.58 ± 0.10	0.63 ± 0.14	0.69 ± 0.33	1.62 ± 0.14	1.76 ± 0.25
Urine	93.26 ± 3.61	94.66 ± 1.88	84.08 ± 2.13	71.16 ± 1.05	58.76 ± 3.44
Muscle	0.02 ± 0.03	0.11 ± 0.18	0.09 ± 0.12	0.02 ± 0.05	0.11 ± 0.18
Pancreas	0.06 ± 0.10	0.32 ± 0.07	3.78 ± 1.09	7.01 ± 1.42	6.89 ± 1.20
Carcass	4.34 ± 2.64	2.73 ± 1.08	2.77 ± 0.75	4.95 ± 1.41	6.69 ± 2.48

Table 19

^{177}Lu-DOTA-8-Aoc-BBN[7-14]NH_2 biodistribution (Avg %ID/gm, n = 5) in PC-3 tumor bearing mice

Time Tissue	1 hr (n = 5)	4 hrs (n = 5)	24 hrs (n = 5)
Blood	0.38 ± 0.22	0.08 ± 0.07	0.01 ± 0.01
Heart	0.15 ± 0.22	0.07 ± 0.13	0.06 ± 0.09
Lung	0.18 ± 0.09	0.11 ± 0.15	0.14 ± 0.26
Liver	0.30 ± 0.05	0.13 ± 0.02	0.03 ± 0.02
Spleen	0.33 ± 0.51	0.60 ± 0.36	0.08 ± 0.10
Stomach	1.38 ± 0.52	0.34 ± 0.34	0.19 ± 0.13
L. Intestine	3.29 ± 0.61	7.29 ± 3.73	1.90 ± 0.53
S. Intestine	5.60 ± 0.46	1.93 ± 0.96	0.48 ± 0.14
Kidney	4.70 ± 0.95	2.18 ± 0.31	0.60 ± 0.20
Muscle	0.11 ± 0.13	0.15 ± 0.21	0.10 ± 0.17
Pancreas	38.53 ± 3.61	22.18 ± 4.66	4.97 ± 2.28
Tumor	4.22 ± 1.09	3.03 ± 0.91	1.54 ± 1.14

TABLE 20.

^{177}Lu-DOTA-8-Aoc-BBN[7-14]NH_2 biodistribution (Avg %ID, n = 5) in PC-3 tumor bearing mice.

Time Tissue	1 hr (n = 5)	4 hrs (n = 5)	24 hrs (n = 5)
Blood	0.62 + 0.44	0.12 + 0.11	0.01 + 0.02
Heart	0.01 + 0.02	0.01 + 0.02	0.01 + 0.01
Lung	0.04 + 0.02	0.05 + 0.09	0.03 + 0.05
Liver	0.38 + 0.09	0.15 + 0.03	0.04 + 0.03
Spleen	0.03 + 0.04	0.05 + 0.02	0.01 + 0.01
Stomach	0.61 + 0.09	0.22 + 0.06	0.09 + 0.06
L. Intestine	3.64 + 0.72	7.28 + 4.23	1.75 + 0.23
S. Intestine	8.20 + 1.72	2.51 + 0.75	0.67 + 0.12
Kidney	1.35 + 0.41	0.61 + 0.08	0.17 + 0.06
Urine	67.41 + 2.45	79.76 + 6.48	85.85 + 1.39
Muscle	0.01 + 0.02	0.02 + 0.03	0.02 + 0.03
Pancreas	9.70 + 1.12	5.23 + 1.68	1.31 + 0.45
Tumor	1.15 + 0.72	0.78 + 0.27	0.29 + 0.18
Carcass	6.18 + 1.01	2.52 + 1.18	2.08 + 3.14

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CLAIMS

What is claimed is:

1. A compound comprising a metal complexed with a chelating group attached to a gastrin releasing peptide (GRP) receptor agonist which includes a bombesin agonist binding moiety.

2. The compound according to claim 1, wherein said compound has a structure of the formula X-Y-B wherein X is a metal chelating group, Y is a spacer group or covalent bond and B is a gastrin releasing peptide receptor agonist which includes a bombesin agonist binding moiety.

3. The compound of claim 2 wherein Y is selected from the group consisting of at least one amino acid residue, a hydrocarbon chain and a combination thereof.

4. The compound of claim 2 wherein X is selected from the group consisting of DOTA, DTPA, S_4, N_3S, N2S2, NS3 and derivatives thereof.

5. The compound of claim 4 wherein Y is selected from the group consisting of at least one amino acid residue, a hydrocarbon chain and a combination thereof and B is selected from the group consisting of BBN(7-14) and BBN(8-14).

6. The compound of claim 4 wherein X is DOTA or a derivative thereof.

7. The compound of claim 6 wherein Y is selected is selected from the group consisting of at least one amino acid residue, a hydrocarbon chain and a combination thereof and B is selected from the group consisting of BBN(7-14) and BBN(8-14).

8. The compound of claim 7 wherein Y is a combination of L-glutamine and a hydrocarbon chain.

9. The compound of claim 8 wherein Y is a combination of L-glutamine and a C1 to C10 hydrocarbon chain.

10. The compound of claim 9 wherein Y is selected from the group consisting of glycine, β-alanine, gamma-aminobutanoic acid, 5-aminovaleric acid (5-Ava), 6-aminohexanoic acid, 7-aminoheptanoic acid, 8-aminooctanoic acid (8-Aoc), 9-aminononanoic acid, 10-aminodecanoic acid and 11-aminoundecanoic acid (11-Aun).

11. The compound of claim 4 wherein X is N_3S or a derivative thereof.

12. The compound of claim 11 wherein Y is selected from the group consisting of at least one amino acid residue, a hydrocarbon chain and a combination thereof and B is selected from the group consisting of BBN(7-14) and BBN(8-14).

13. The compound of claim 12 wherein Y is gly-ser-gly.

14. A complex comprising a metal and a compound having a structure of the formula X-Y-B wherein X is a metal chelating group, Y is a spacer group or covalent bond and B is a gastrin releasing peptide receptor agonist which includes a bombesin agonist binding moiety.

15. The complex of claim 14 wherein the metal is selected from the group consisting of transition metals, lanthanides, auger-electron emitting isotopes, and α-, β- or γ-emitting isotopes.

16. The complex of claim 14 wherein the metal is selected from the group consisting of: 105Rh-, ^{99m}Tc-, $^{186/188}$Re-, ^{153}Sm-, ^{166}Ho-, ^{111}In-, ^{90}Y-, ^{177}Lu-, ^{149}Pm-, ^{166}Dy-, ^{175}Yb-, ^{199}Au- and ^{117}mSn-.

17. The complex of claim 16 wherein X is selected from the group consisting of DOTA, DTPA, S_4, N_3S, N2S2, NS3 and derivatives thereof.

18. The complex of claim 17 wherein Y is selected from the group consisting of at least one amino acid residue, a hydrocarbon chain and a combination thereof and B is selected from the group consisting of BBN(7-14) and BBN(8-14).

19. The complex of claim 16 wherein X is DOTA or a derivative thereof.

20. The complex of claim 19 wherein Y is selected is selected from the group consisting of at least one amino acid residue, a hydrocarbon chain and a combination thereof and B is selected from the group consisting of BBN(7-14) and BBN(8-14).

21. The complex of claim 20 wherein Y is a combination of L-glutamine and a hydrocarbon chain.

22. The complex of claim 21 wherein Y is a combination of L-glutamine and a C1 to C10 hydrocarbon chain.

23. The complex of claim 22 wherein Y is selected from the group consisting of glycine, β-alanine, gamma-aminobutanoic acid, 5-aminovaleric acid (5-Ava), 6-aminohexanoic acid, 7-aminoheptanoic acid, 8-aminooctanoic acid (8-Aoc), 9-aminononanoic acid, 10-aminodecanoic acid and 11-aminoundecanoic acid (11-Aun).

24. The complex of claim 23 wherein Y is 8-aminooctanoic acid.

25. The complex of claim 23 consisting of ^{90}Y-DOTA-8-Aoc-BBN(7-14)NH_2.

26. The complex of claim 23 consisting of ^{111}In-DOTA-8-Aoc-BBN(7-14) NH_2.

27. The complex of claim 23 consisting of ^{177}Lu-DOTA-8-Aoc-BBN(7-14) NH_2.

28. The complex of claim 23 consisting of ^{149}Pm-DOTA-8-Aoc-BBN(7-14) NH_2.

29. The complex of claim 23 consisting of ^{90}Y-DOTA-5-Ava-BBN(7-14)NH_2.

30. The complex of claim 23 consisting of ^{111}In-DOTA-5-Ava-BBN(7-14) NH_2.

31. The complex of claim 23 consisting of ^{177}Lu-DOTA-5-Ava-BBN(7-14) NH_2.

32. The complex of claim 23 consisting of ^{149}Pm-DOTA-5-Ava-BBN(7-14) NH_2.

33. The complex of claim 16 wherein X is N_3S or a derivative thereof.

34. The complex of claim 33 wherein Y is selected from the group consisting of at least one amino acid residue, a hydrocarbon chain and a combination thereof and B is selected from the group consisting of BBN(7-14) and BBN(8-14).

35. The complex of claim 34 wherein Y is gly-ser-gly.

36. The complex of claim 34 consisting of ^{99m}Tc-N_3S-gly-ser-gly-BBN(7-14)NH_2.

37. A method of treating a patient using radioisotope therapy by administering an effective amount of a pharmaceutical comprising a metal complex with a chelating group with a gastrin releasing peptide receptor agonist which includes a bombesin agonist moiety.

38. The method according to claim 37, wherein said method includes administering an effective amount of a complex comprising a metal and a compound having a structure of the formula

X-Y-B

wherein X is a metal chelating group, Y is a spacer group or covalent bond and B is a gastrin releasing peptide receptor agonist which includes a bombesin agonist binding moiety.

39. The method of claim 38 wherein the metal is selected from the group consisting of transition metals, lanthanides, auger-electron emitting isotopes, and α-, β- or γ-emitting isotopes.

40. The method of claim 38 wherein the metal is selected from the group consisting of: ^{105}Rh-, ^{99m}Tc-, $^{186/188}$Re-, ^{153}Sm-, ^{166}Ho-, ^{111}In-, ^{90}Y-, ^{177}Lu-, ^{149}Pm-, ^{166}Dy-, ^{175}Yb-, ^{199}Au- and ^{117}mSn-.

41. The method of claim 40 wherein X is selected from the group consisting of DOTA, DTPA, S_4, N_3S, N2S2, NS3 and derivatives thereof.

42. The method of claim 41 wherein X is DOTA or a derivative thereof.

43. The method of claim 42 wherein Y is selected from the group consisting of at least one amino acid residue, a hydrocarbon chain and a combination thereof and B is selected from the group consisting of BBN(7-14) and BBN(8-14).

44. The method of claim 43 wherein Y is a combination of L-glutamine and a hydrocarbon chain.

45. The method of claim 44 wherein Y is selected from the group consisting of glycine, β-alanine, gamma-aminobutanoic acid, 5-aminovaleric acid (5-Ava), 6-aminohexanoic acid, 7-aminoheptanoic acid, 8-aminooctanoic acid (8-Aoc), 9-aminononanoic acid, 10-aminodecanoic acid and 11-aminoundecanoic acid (11-Aun).

46. A method of imaging a patient by administering to a subject a diagnostically effective amount of a compound as set forth in claim 1.

47. The method of claim 46, wherein said method includes administering an effective amount of a complex comprising a metal and a compound having a structure of the formula

X-Y-B

wherein X is a metal chelating group, Y is a spacer group or covalent bond and B is a gastrin releasing peptide receptor agonist which includes a bombesin agonist binding moiety.

48. The method of claim 47 wherein the metal is selected from the group consisting of transition metals, lanthanides, auger-electron emitting isotopes, and α-, β- or γ-emitting isotopes.

49. The method of claim 48 wherein X is selected from the group consisting of DOTA, DTPA, S_4, N_3S, N2S2, NS3 and derivatives thereof.

50. The method of claim 49 wherein X is N_3S or a derivative thereof.

51. The method of claim 50 wherein Y is selected is selected from the group consisting of at least one amino acid residue, a hydrocarbon chain and a combination thereof and B is selected from the group consisting of BBN(7-14) and BBN(8-14).

52. The method of claim 51 wherein Y is gly-ser-gly.

53. A method of forming a therapeutic or diagnostic compound comprising the step of reacting a metal complexed with a chelating group with a gastrin releasing peptide receptor agonist which includes a bombesin agonist moiety.

54. The method of claim 53, wherein said method includes reacting a metal with a compound having a structure of the formula

X-Y-B

wherein X is a metal chelating group, Y is a spacer group or covalent bond and B is a gastrin releasing peptide receptor agonist which includes a bombesin agonist binding moiety.

55. The method of claim 54 wherein the metal is selected from the group consisting of transition metals, lanthanides, auger-electron emitting isotopes, and α-, β- or γ-emitting isotopes.

56. The method of claim 54 wherein the metal is selected from the group consisting of: ^{99m}Tc- and $^{186/188}$Re-.

57. The method of claim 56 wherein Y is selected is selected from the group consisting of at least one amino acid residue, a hydrocarbon chain and a combination thereof.

58. The method of claim 57 wherein X is selected from the group consisting of DOTA, DTPA, S_4, N_3S, N2S2, NS3 and derivatives thereof.

59. The method of claim 58 wherein B is selected from the group consisting of BBN(7-14) and BBN(8-14).

60. The method of claim 59 wherein X is DOTA or a derivative thereof and Y is selected from the group consisting of glycine, β-alanine, gamma-aminobutanoic acid, 5-aminovaleric acid (5-Ava), 6-aminohexanoic acid, 7-aminoheptanoic acid, 8-aminooctanoic acid (8-Aoc), 9-aminononanoic acid, 10-aminodecanoic acid and 11-aminoundecanoic acid (11-Aun).

61. The method of claim 59 wherein X is N_3S or a derivative thereof and Y is gly-ser-gly.

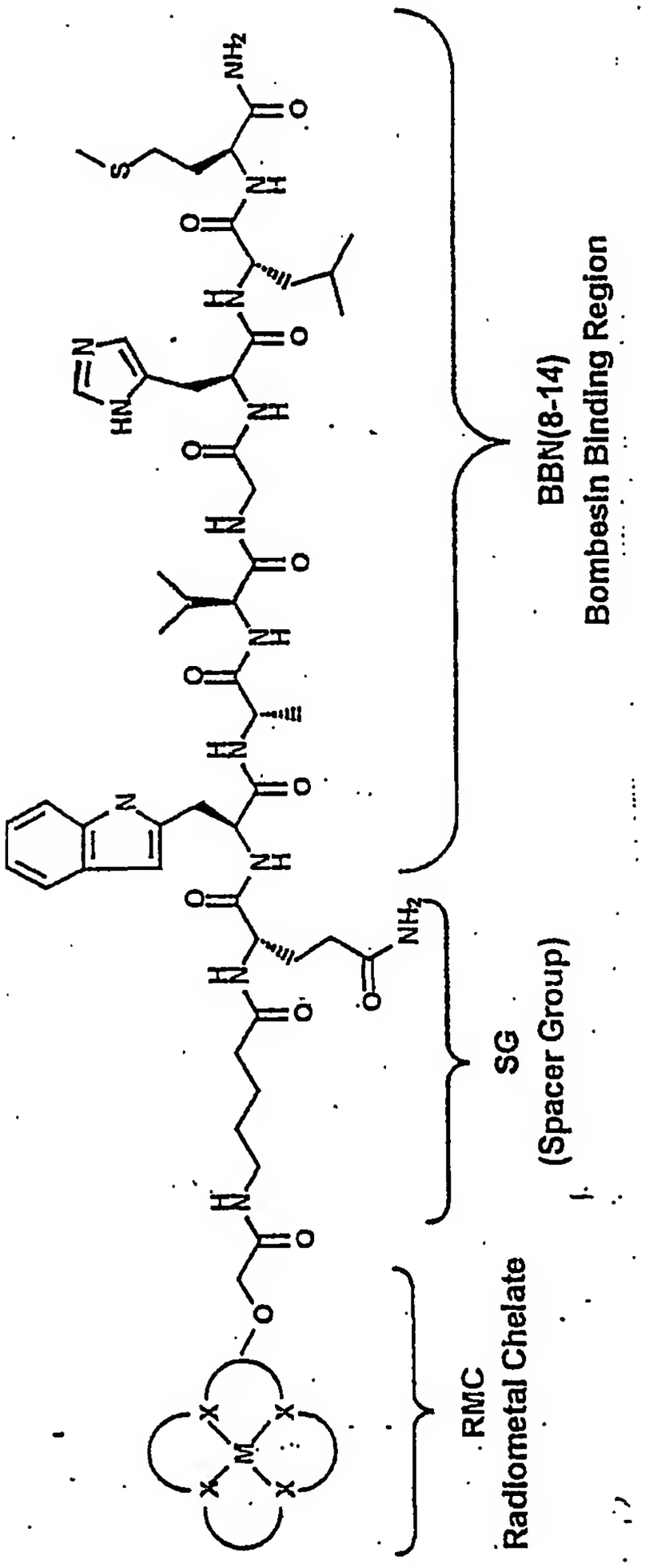
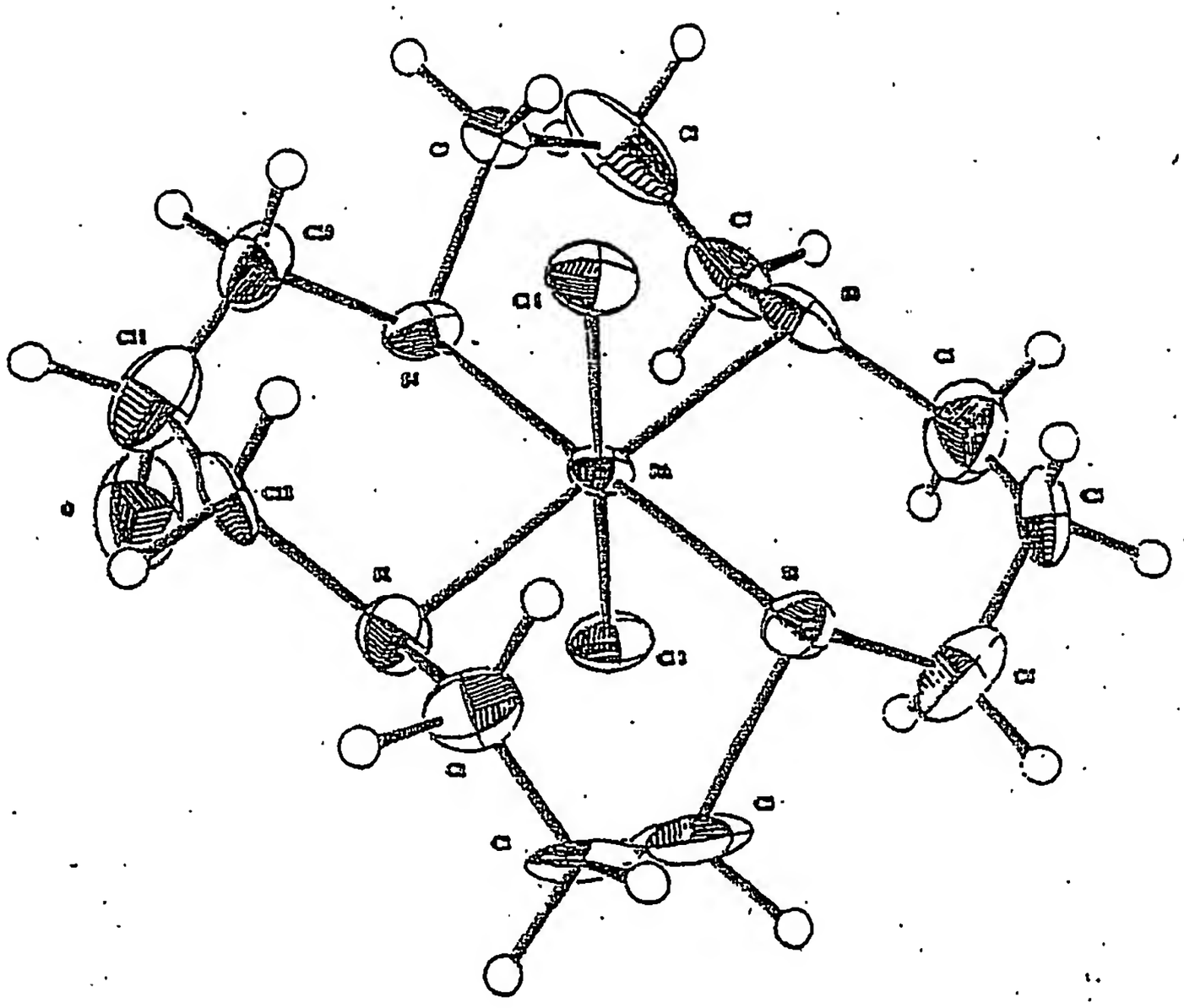

BBN(8-14)
Bombesin Binding Region
SG
(Spacer Group)
RMC
Radiometal Chelate

Figure 1



Figu

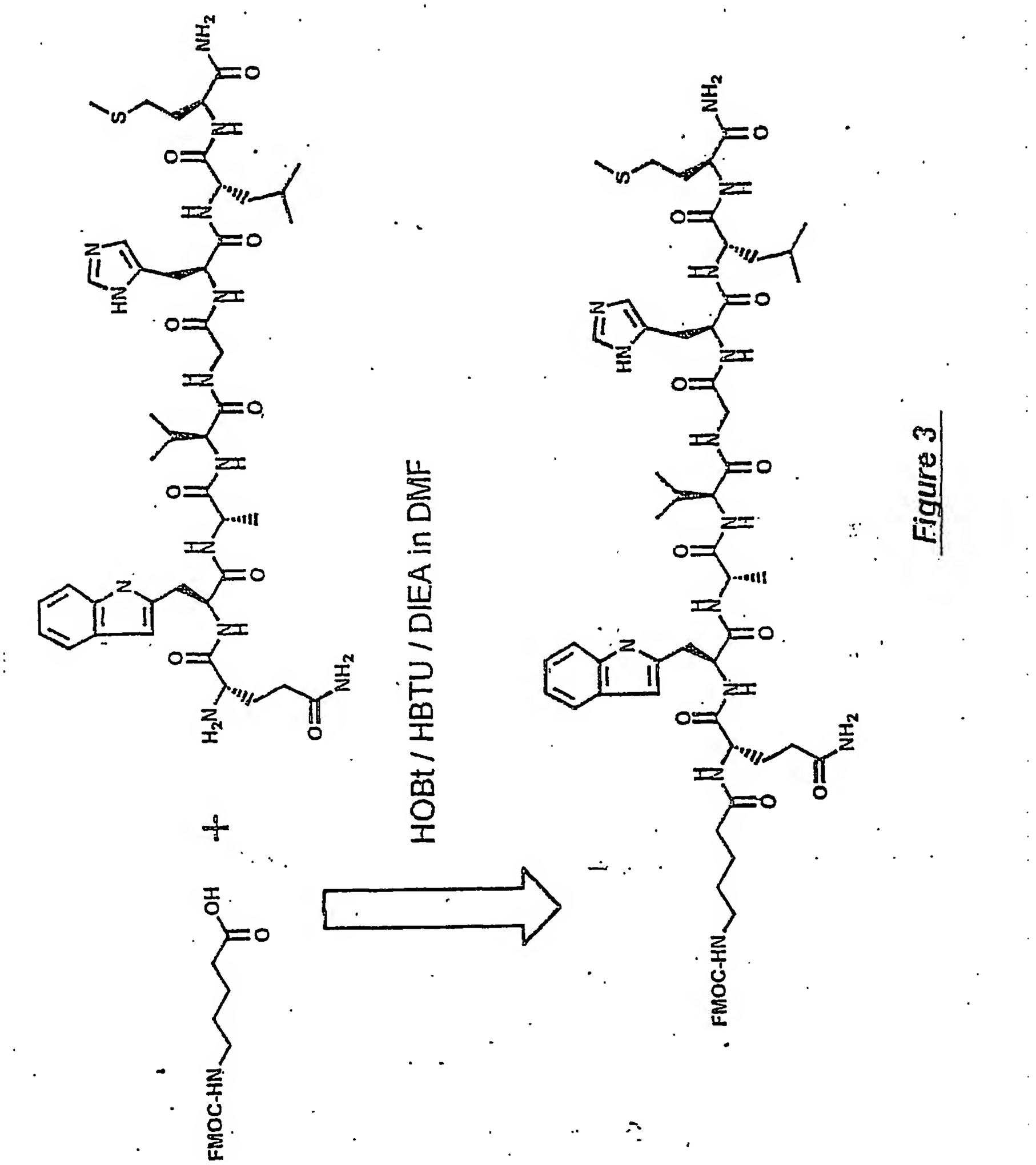


Figure 3

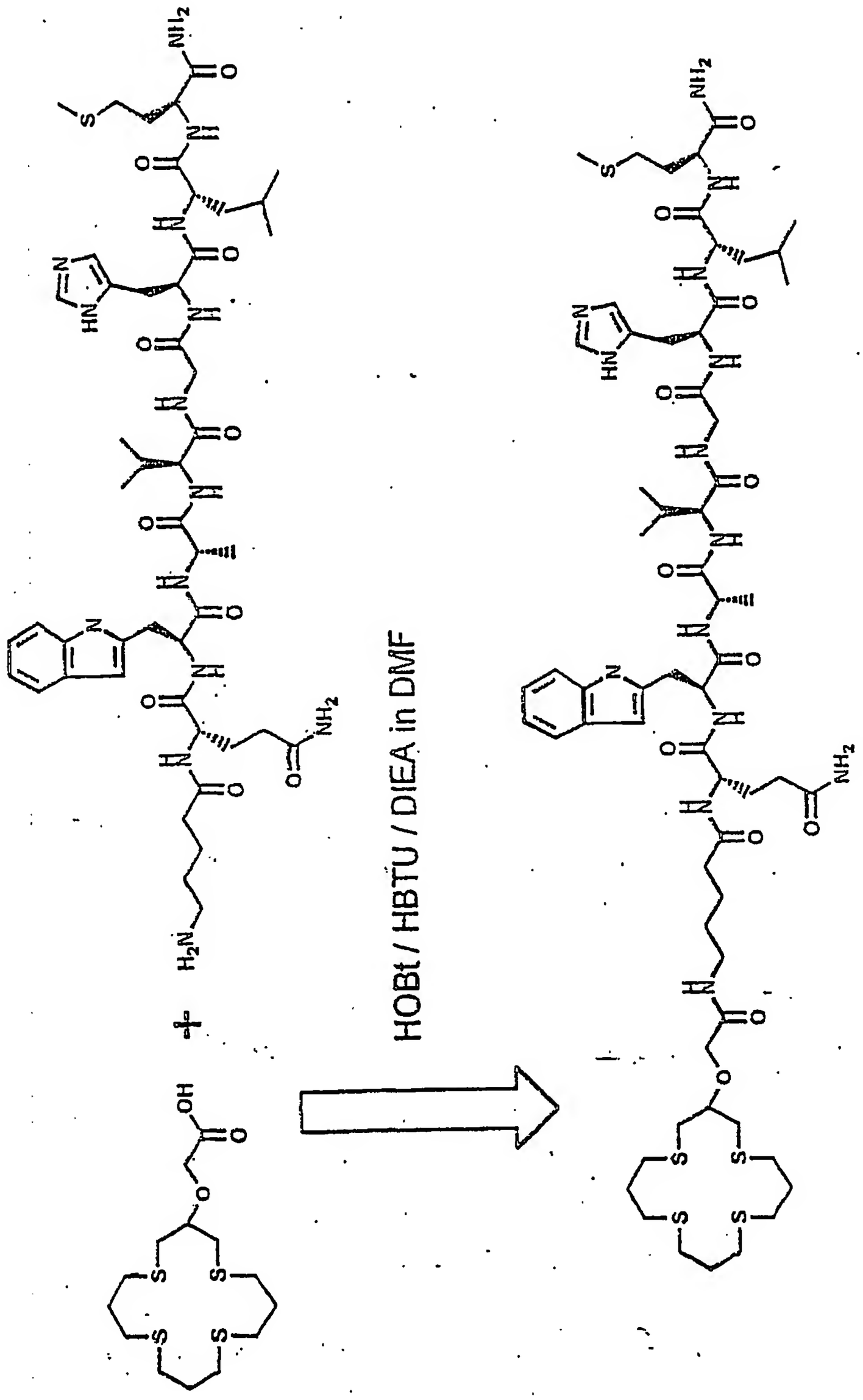


Figure 4

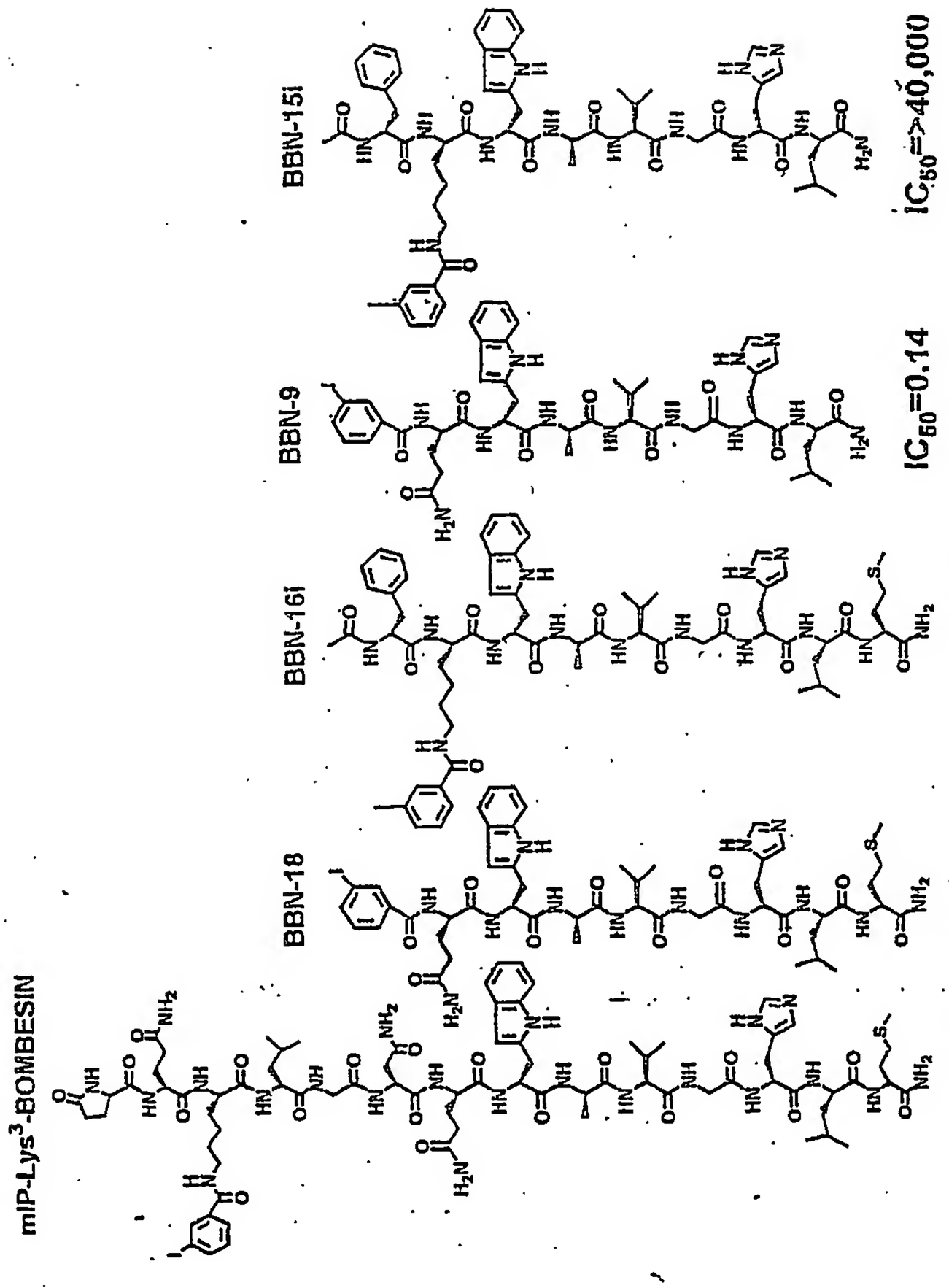

BBN-15i
IC50=>40,000
BBN-9
IC50=0.14
BBN-16i
IC50=18826
BBN-18
IC50=5.80
mIP-Lys3-BOMBESIN
IC50=2.13

Figure 5

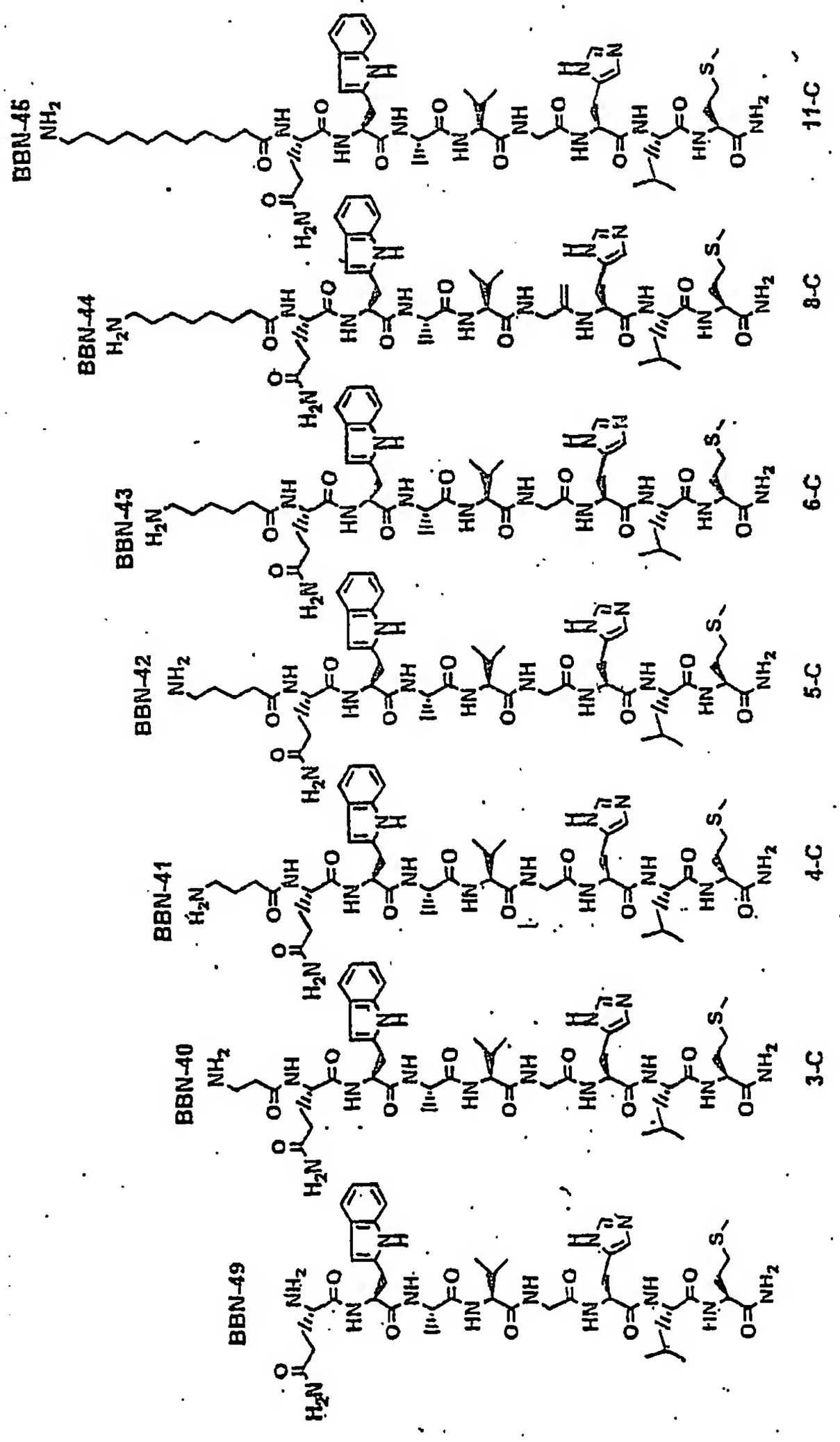


Figure 6

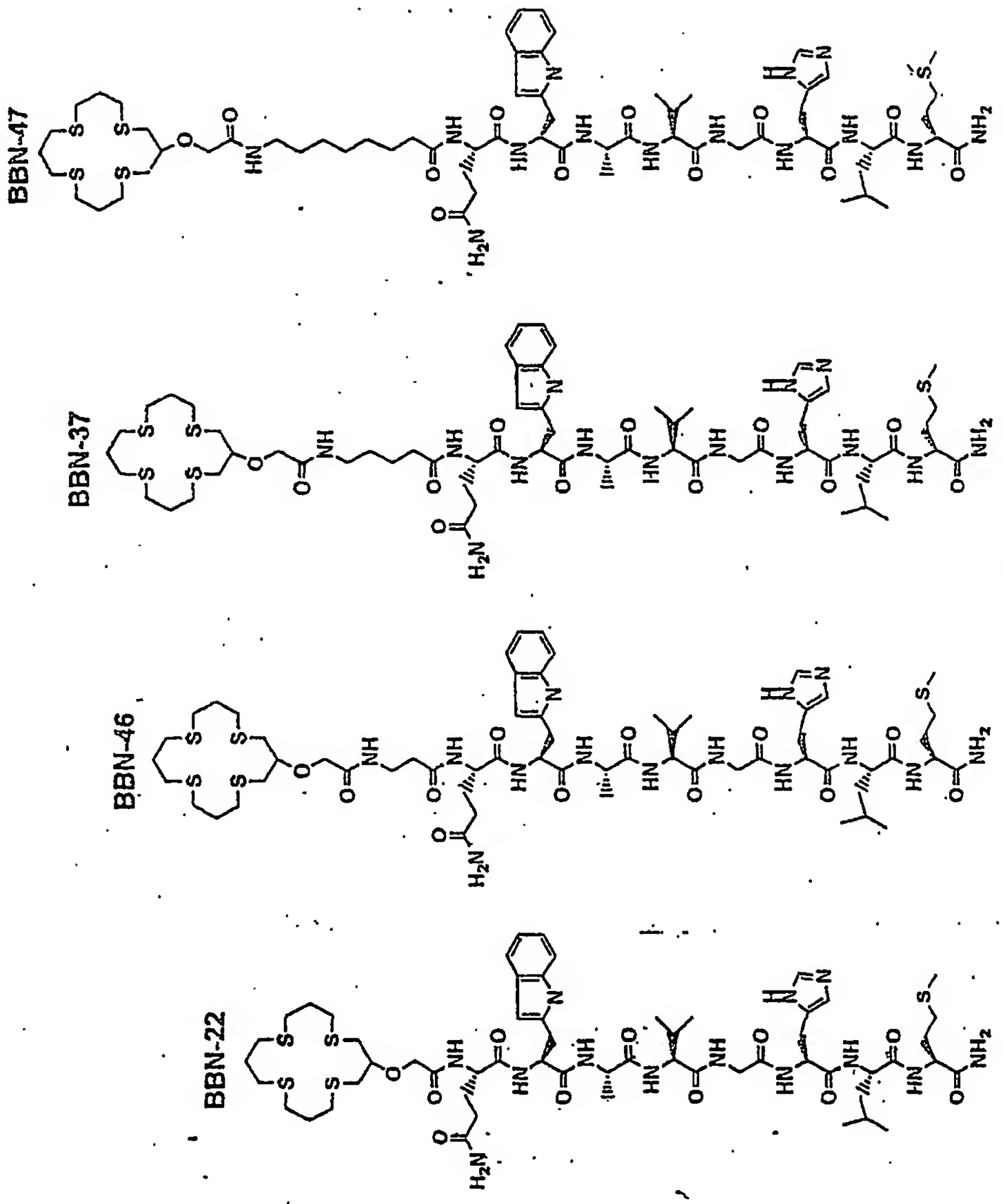


Figure 7

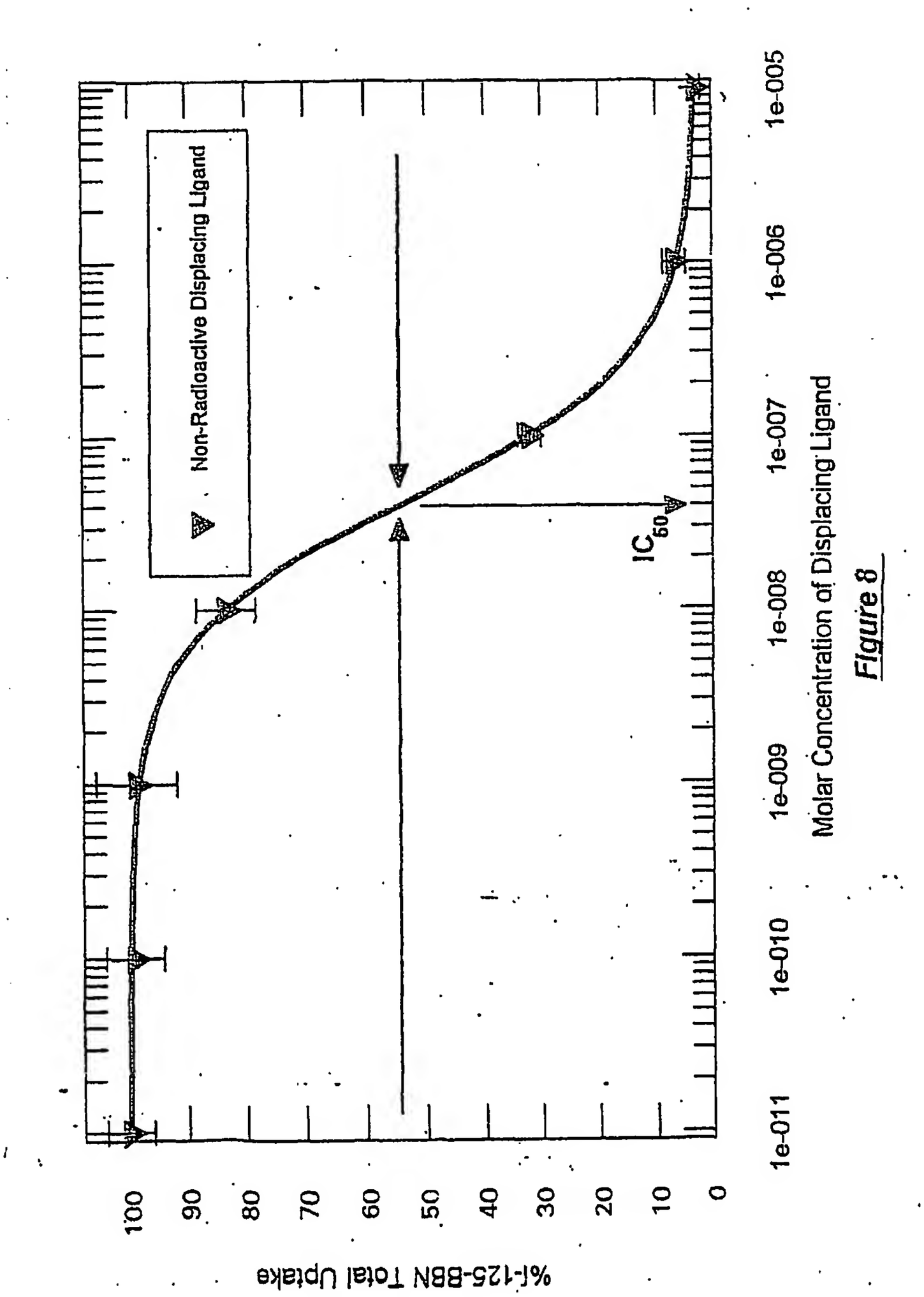

Non-Radioactive Displacing Ligand
IC_{50}
%I-125-BBN Total Uptake
100
90
80
70
60
50
40
30
20
10
0
1e-009
1e-008
1e-007
1e-006
1e-005
1e-010
1e-011
Molar Concentration of Displacing Ligand

Figure 8

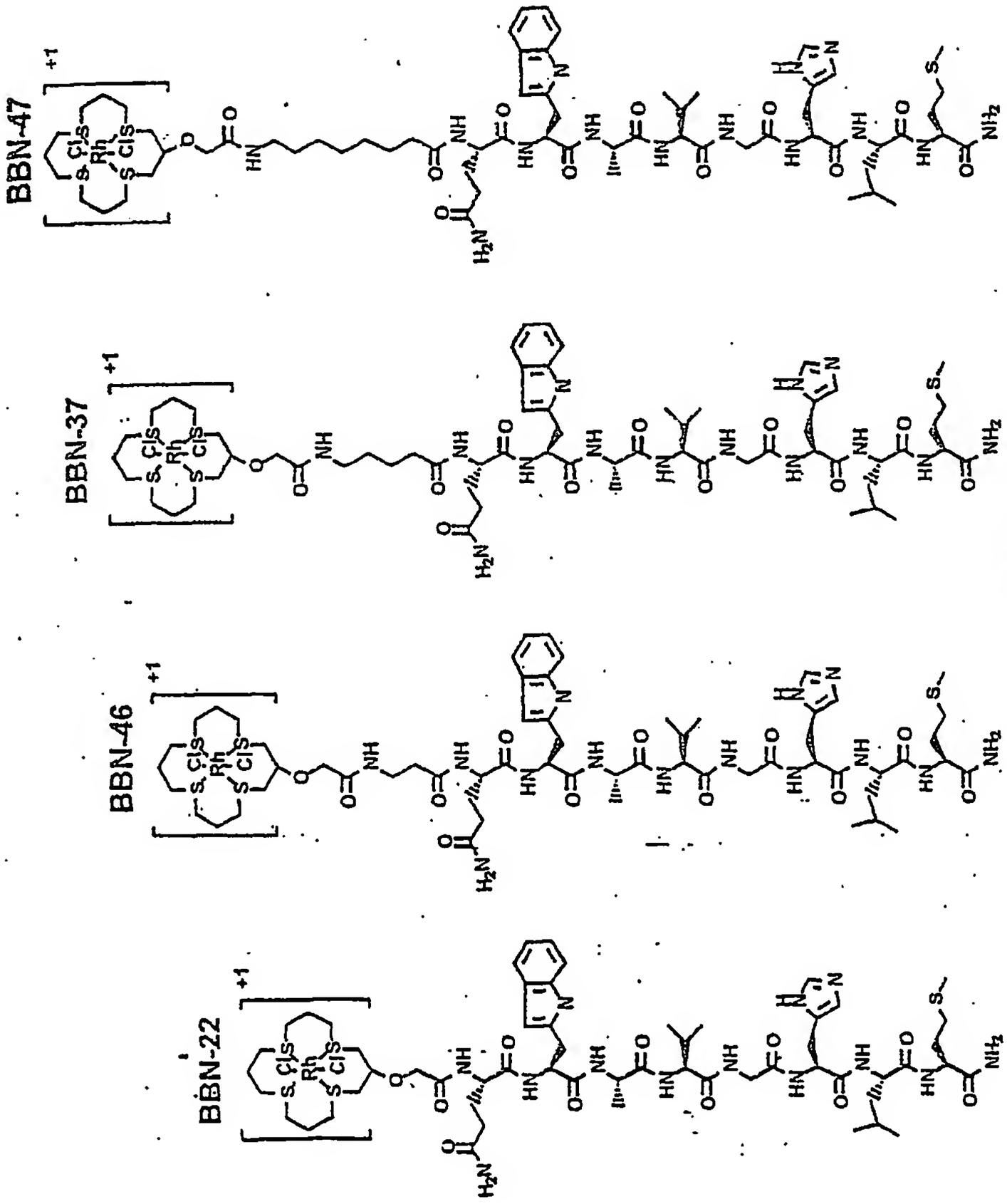


Figure 9

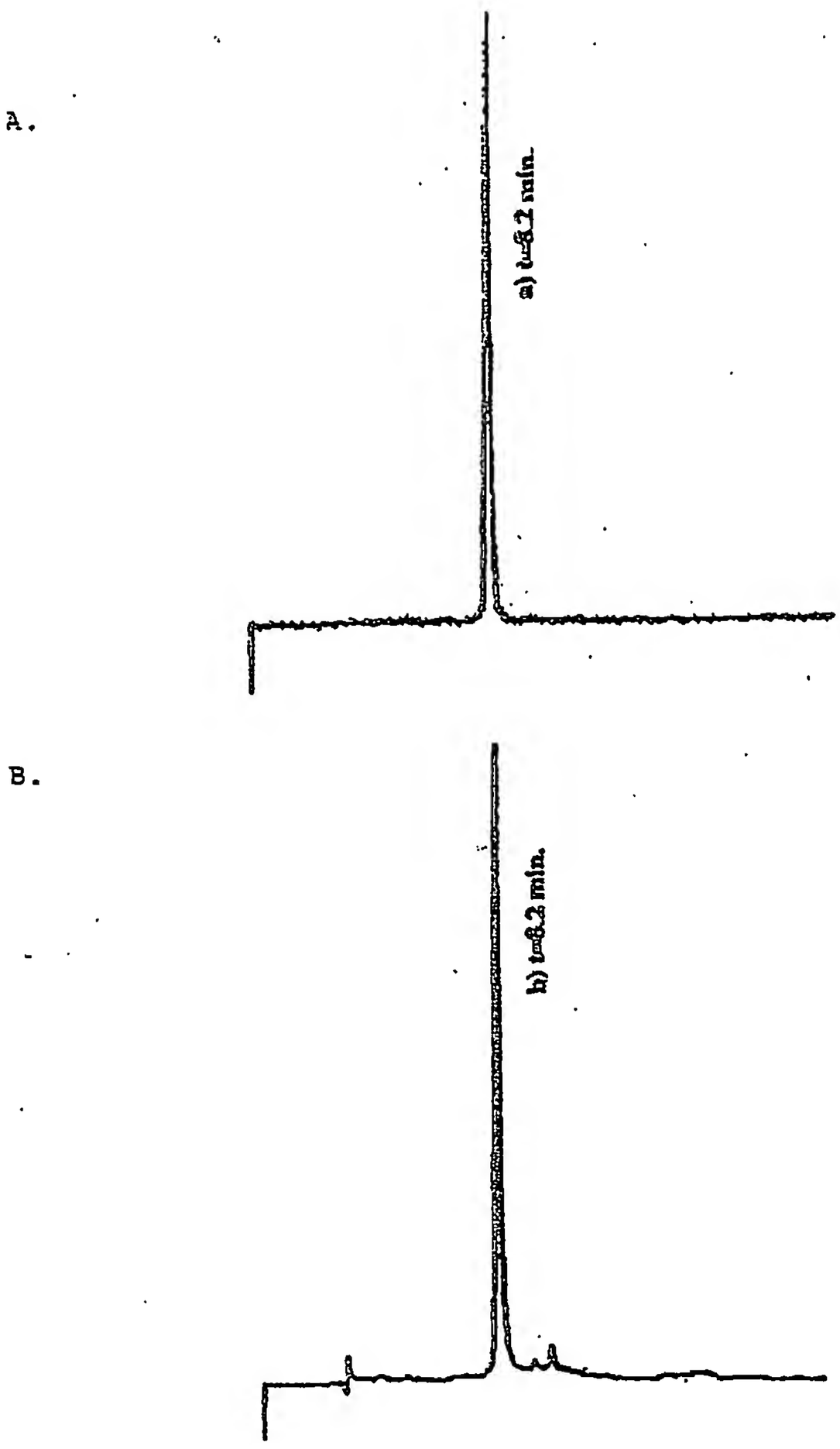


Figure 10

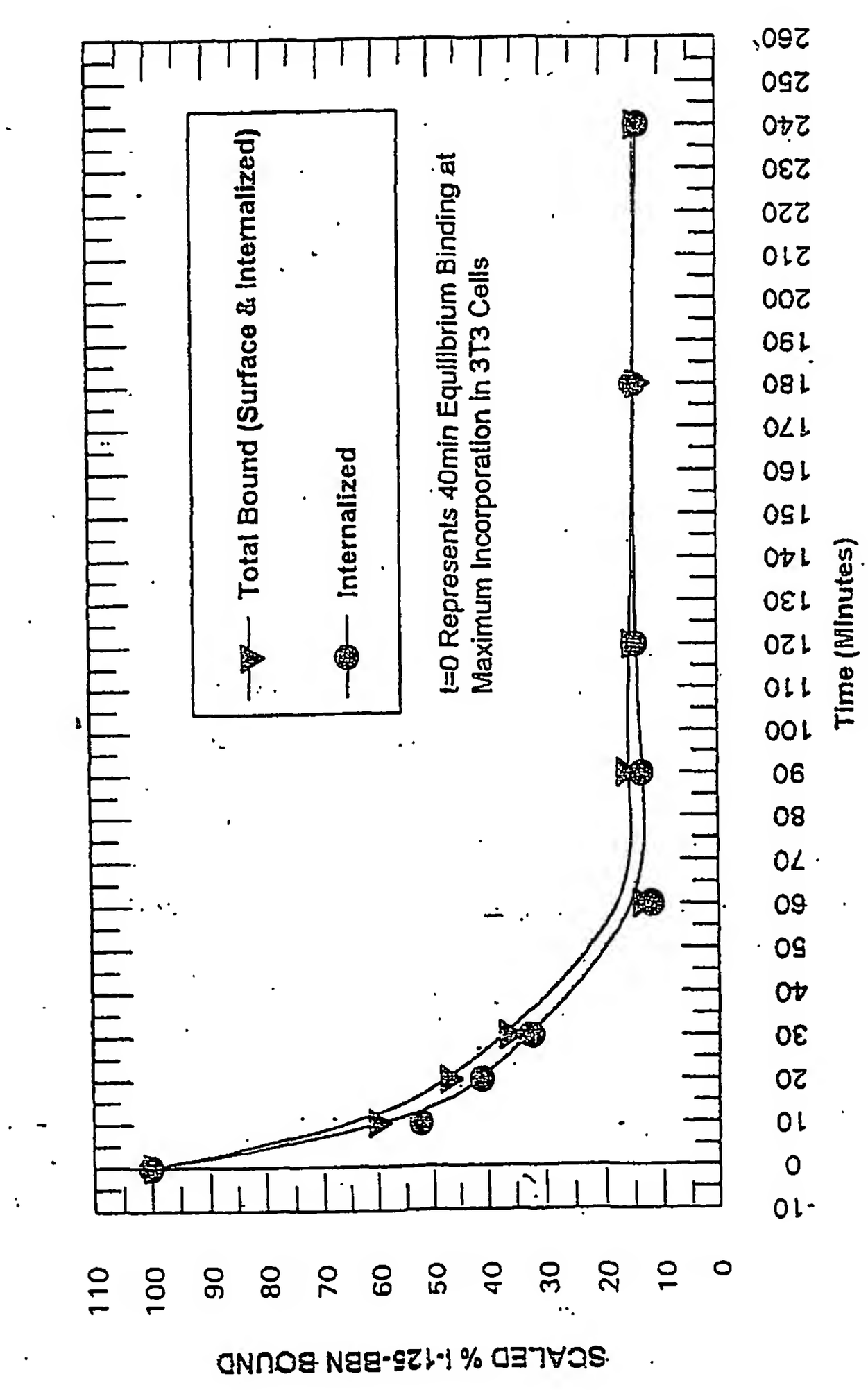

Total Bound (Surface & Internalized)
Internalized
t=0 Represents 40min Equilibrium Binding at Maximum Incorporation in 3T3 Cells
SCALED % I-125-BRN BOUND
Time (Minutes)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260

Figure 11

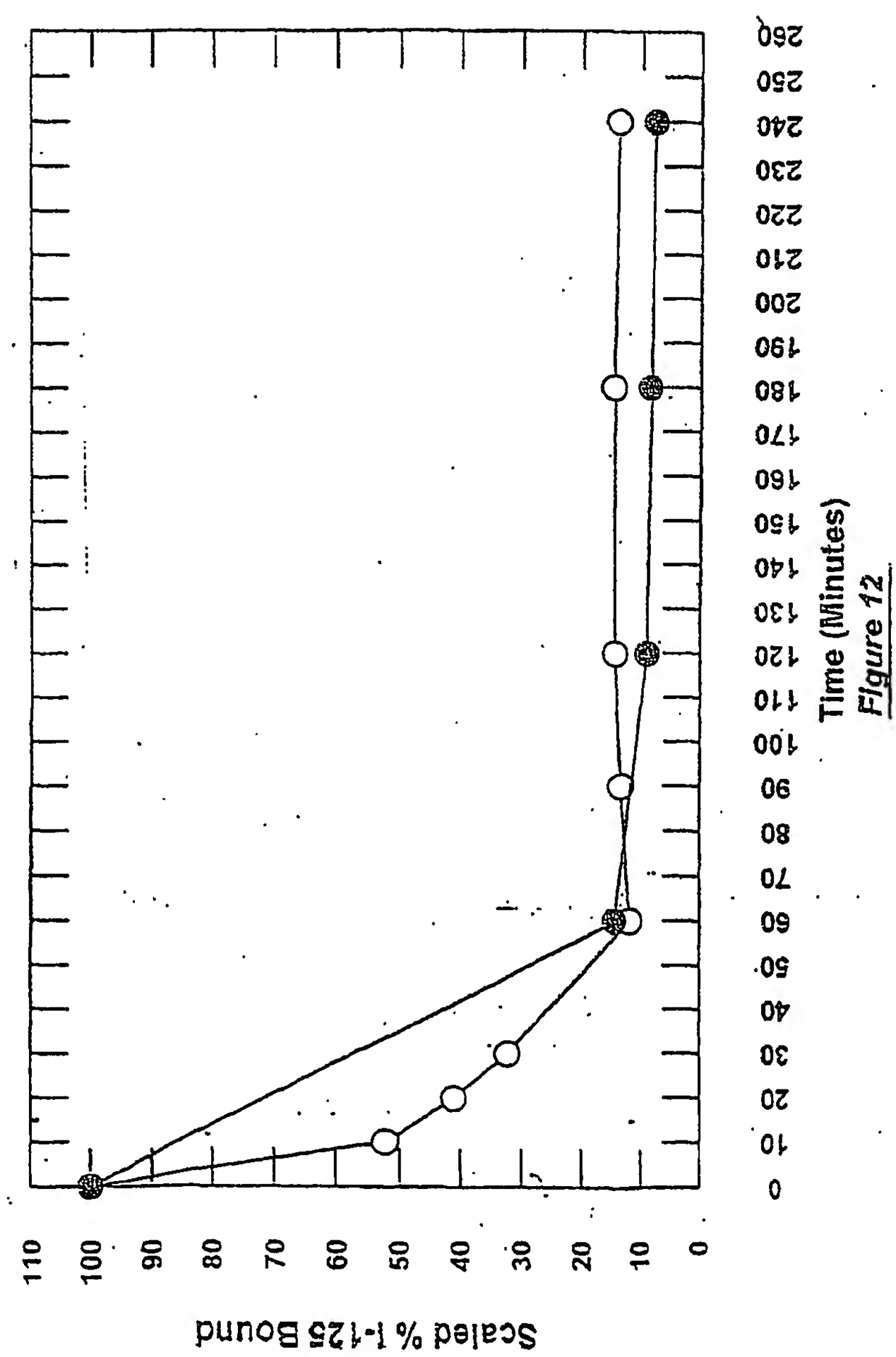

Scaled % I-125 Bound
110
100
90
80
70
60
50
40
30
20
10
0
Time (Minutes)
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

Figure 12

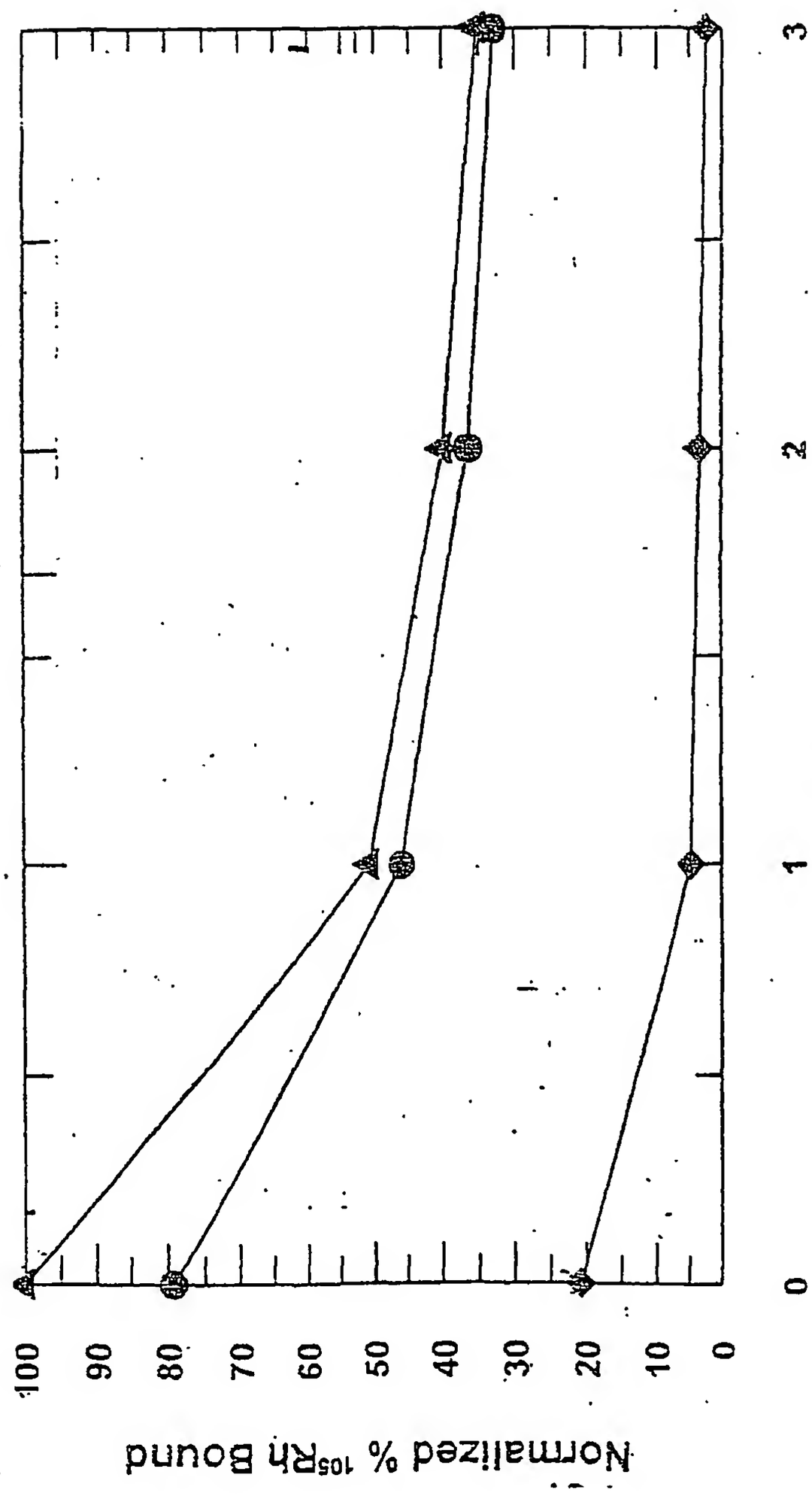

Normalized % 105Rh Bound
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
Time (Hours)

Figure 13

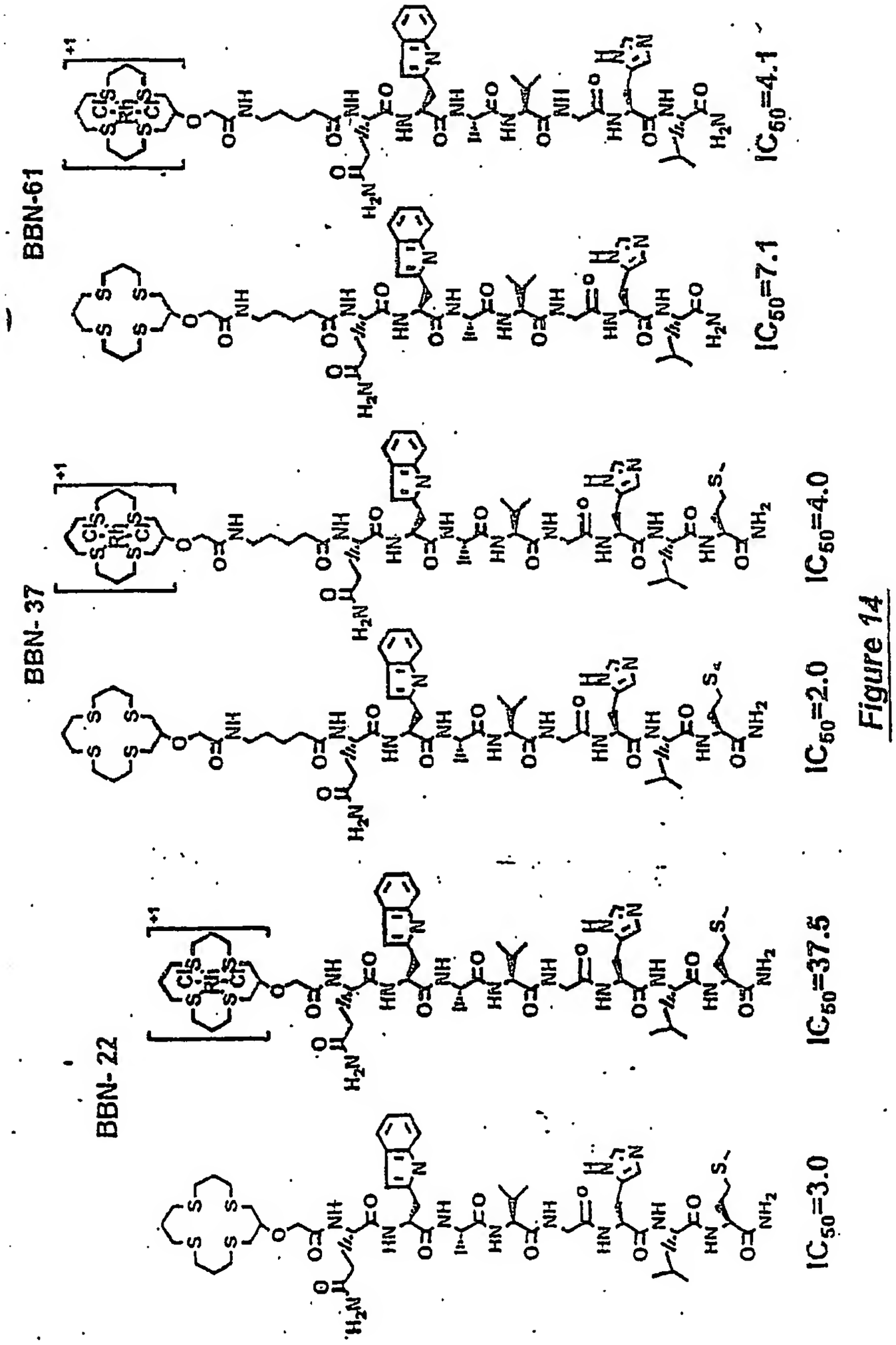

BBN-61
IC50=4.1
IC50=7.1
BBN-37
IC50=2.0
IC50=4.0
BBN-22
IC50=37.5
IC50=3.0

Figure 14

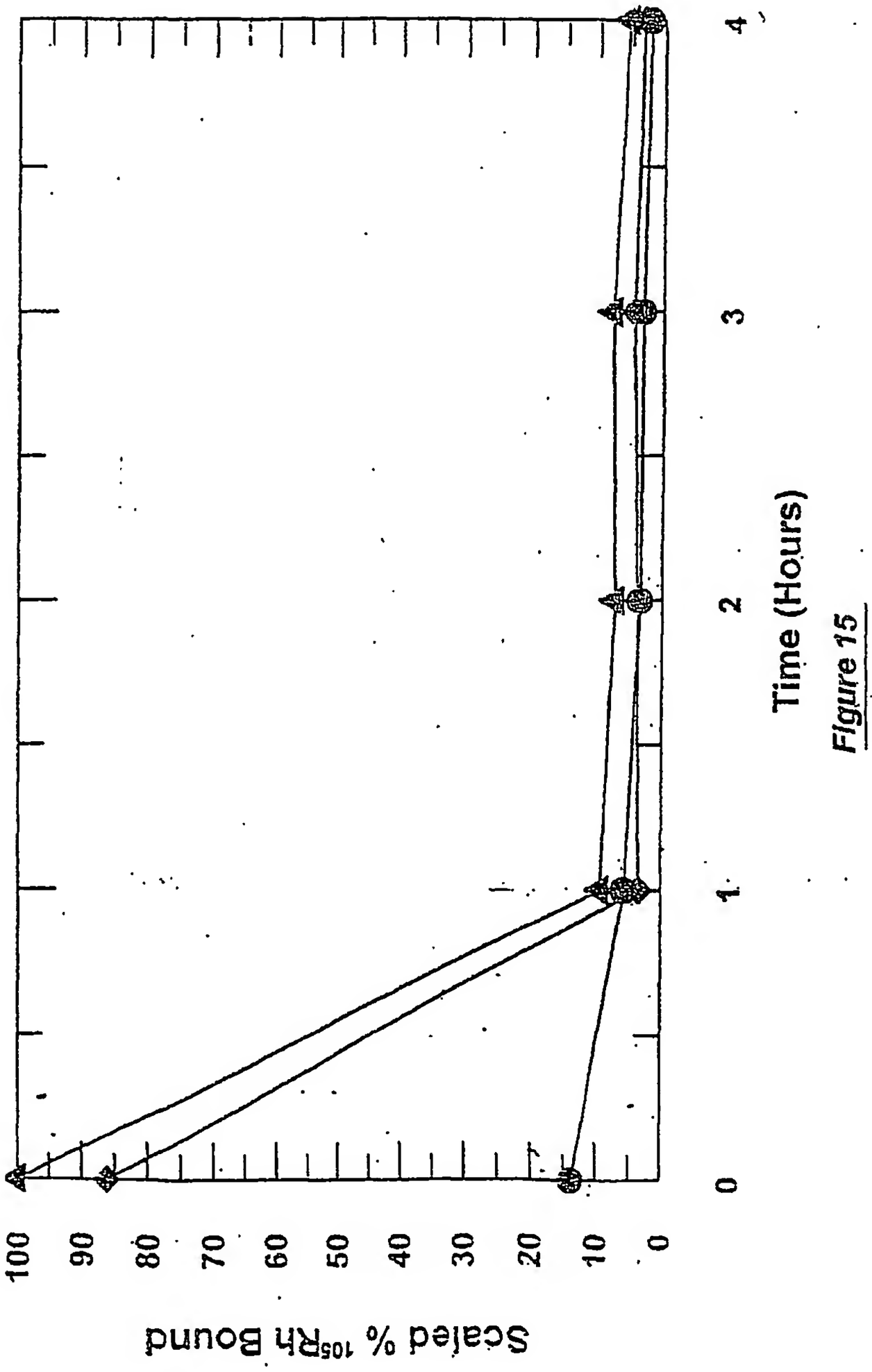

Scaled % 105Rh Bound
100
90
80
70
60
50
40
30
20
10
0
Time (Hours)
0
1
2
3
4

Figure 15

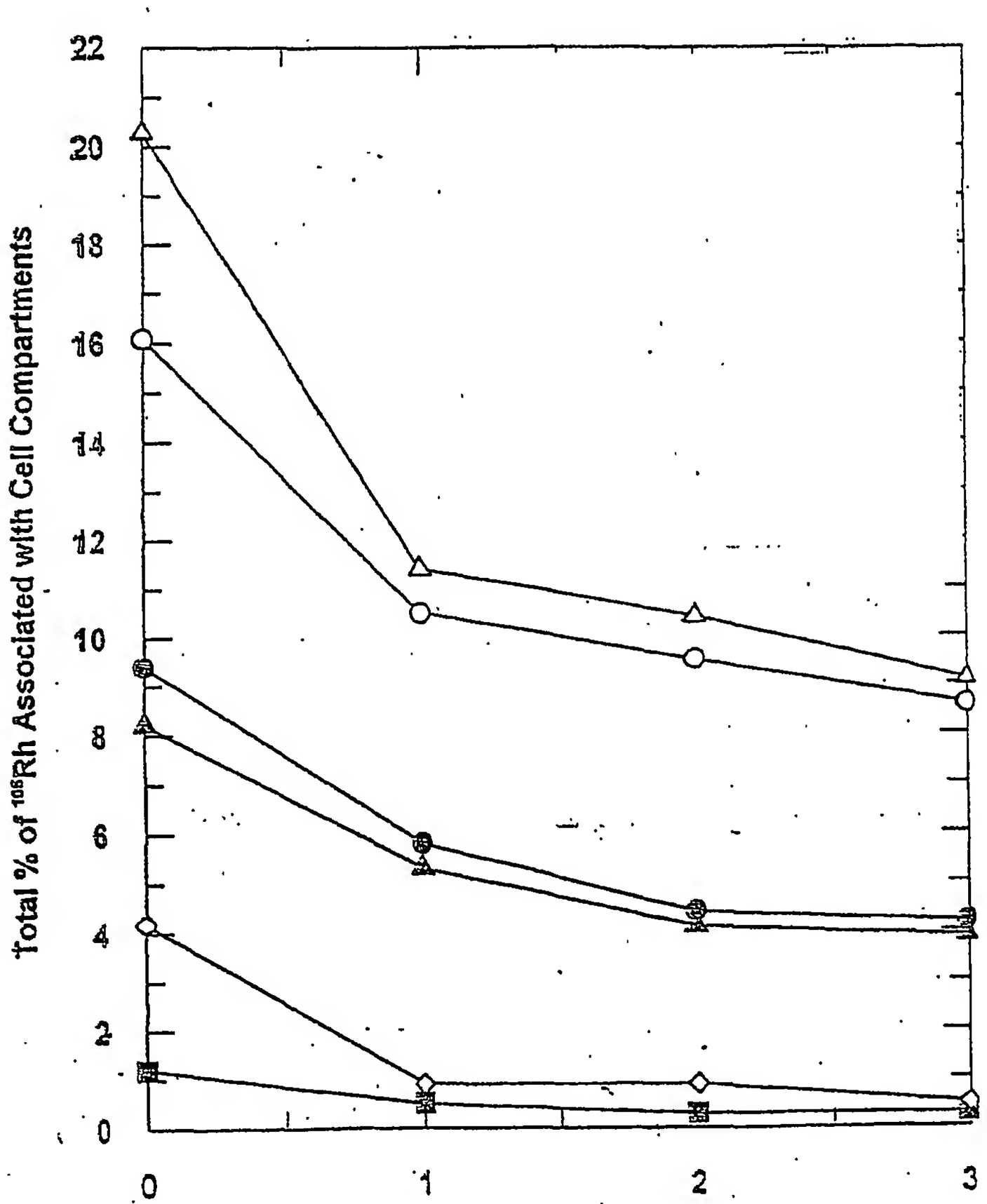
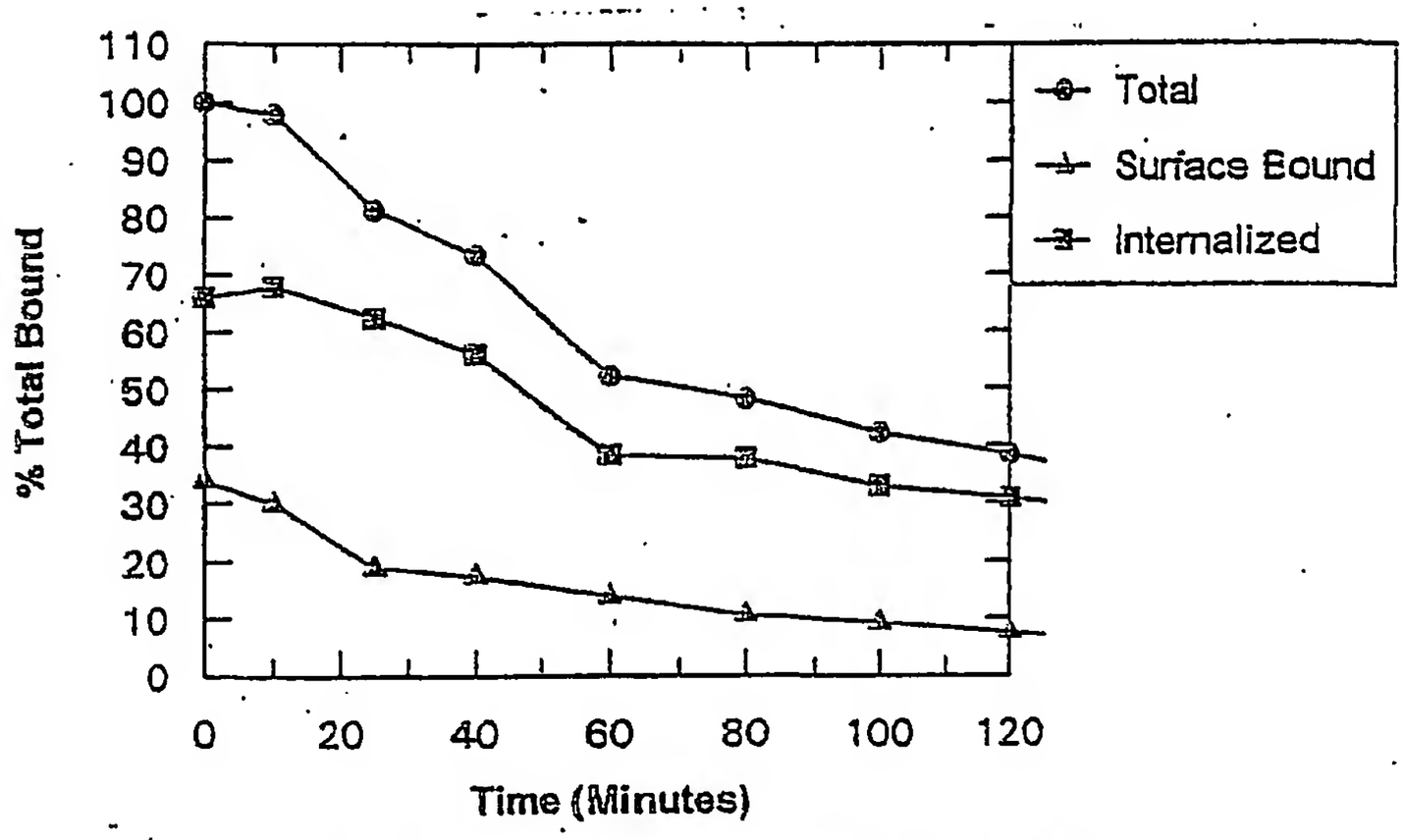

Total % of 186Rh Associated with Cell Compartments
0
2
4
6
8
10
12
14
16
18
20
22
0
1
2
3
Time (Hours)

Figure 16

A.


% Total Bound
110
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Time (Minutes)
Total
Surface Bound
Internalized

B.

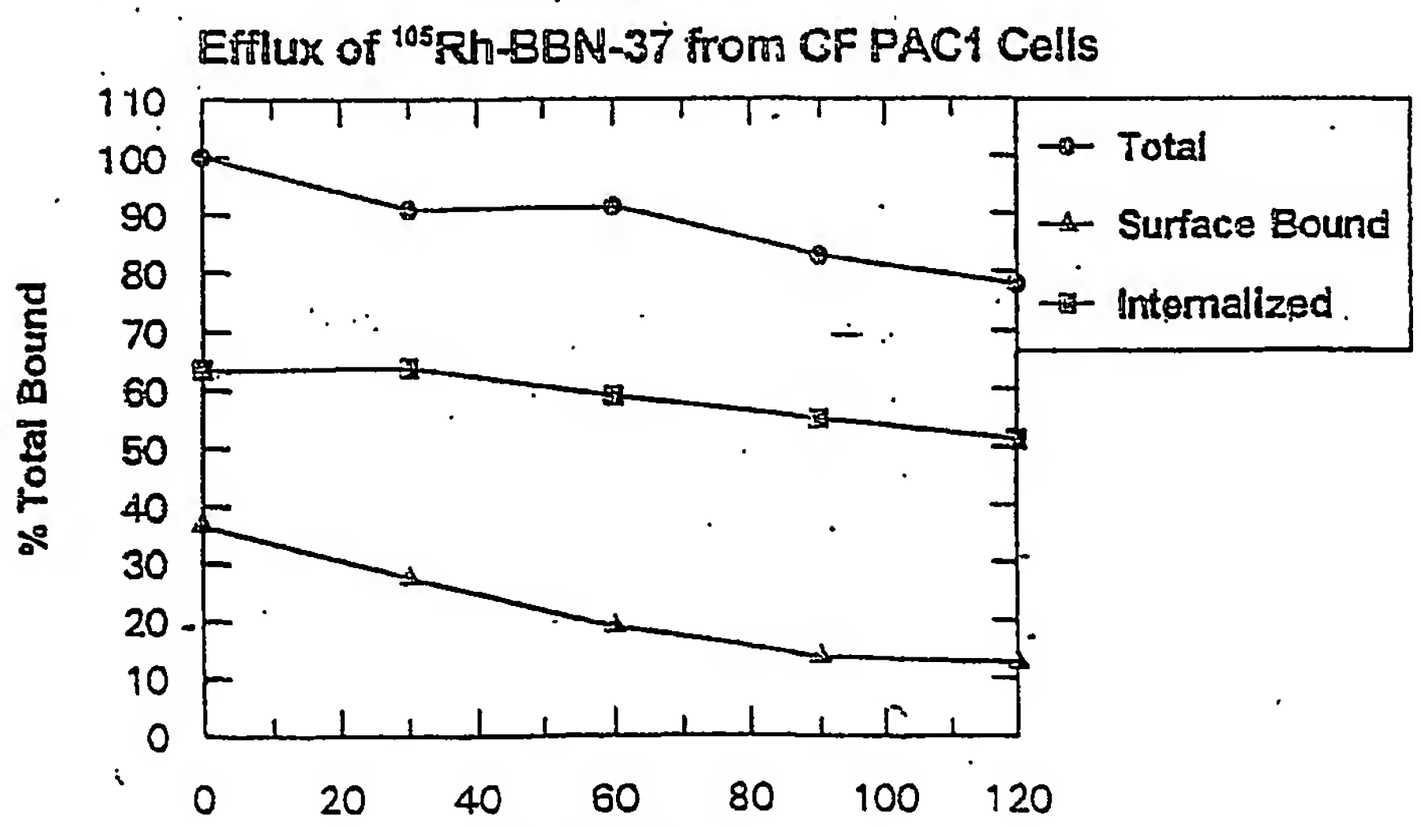
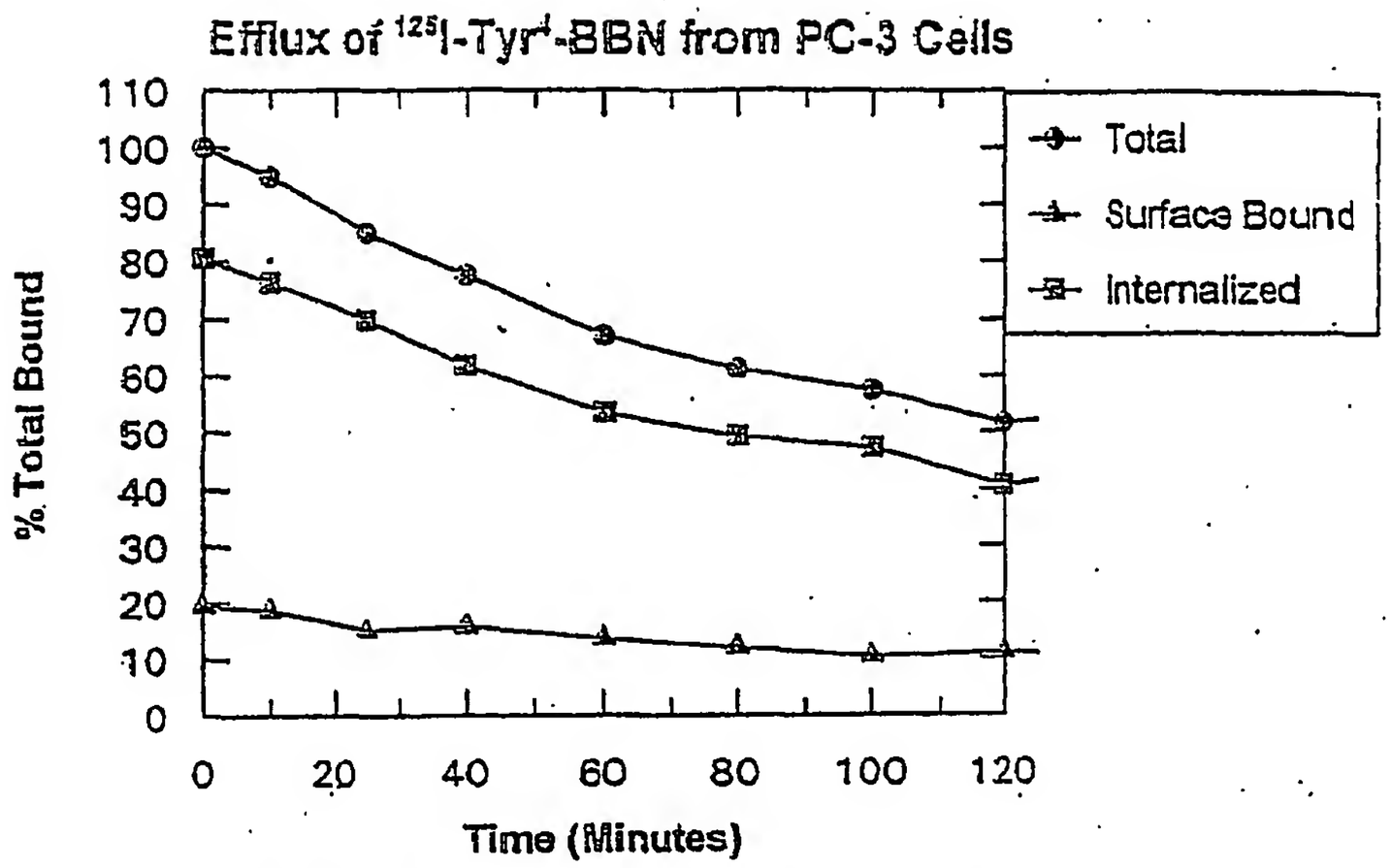

Efflux of 105Rh-BBN-37 from CF PAC1 Cells
% Total Bound
110
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Time (Minutes)
Total
Surface Bound
Internalized

Figure 17

A.


Efflux of ^{125}I-Tyr^4-BBN from PC-3 Cells
% Total Bound
110
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Time (Minutes)
Total
Surface Bound
Internalized

B.

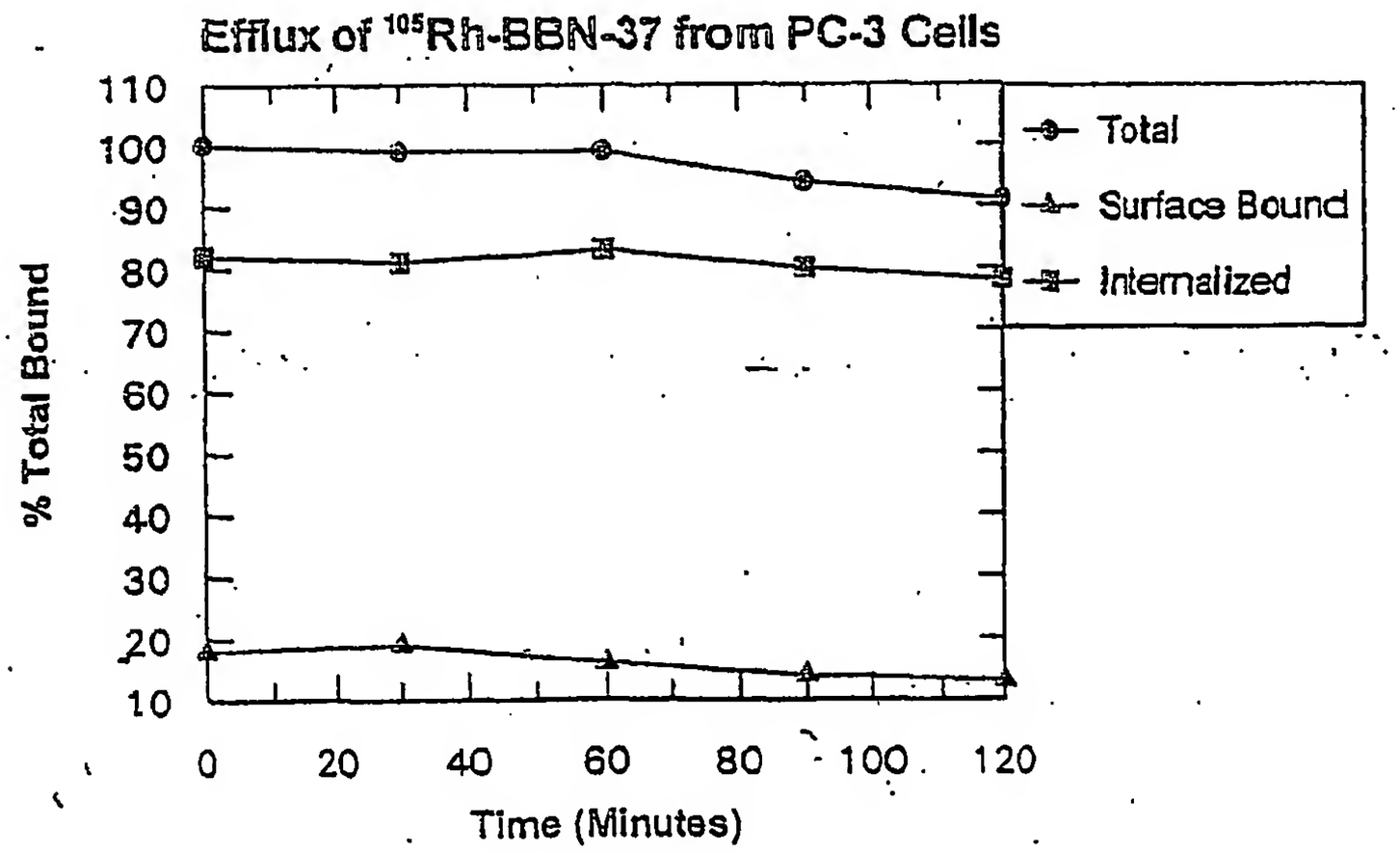

Efflux of ^{105}Rh-BBN-37 from PC-3 Cells
% Total Bound
110
100
90
80
70
60
50
40
30
20
10
0
20
40
60
80
100
120
Time (Minutes)
Total
Surface Bound
Internalized

Figure 18

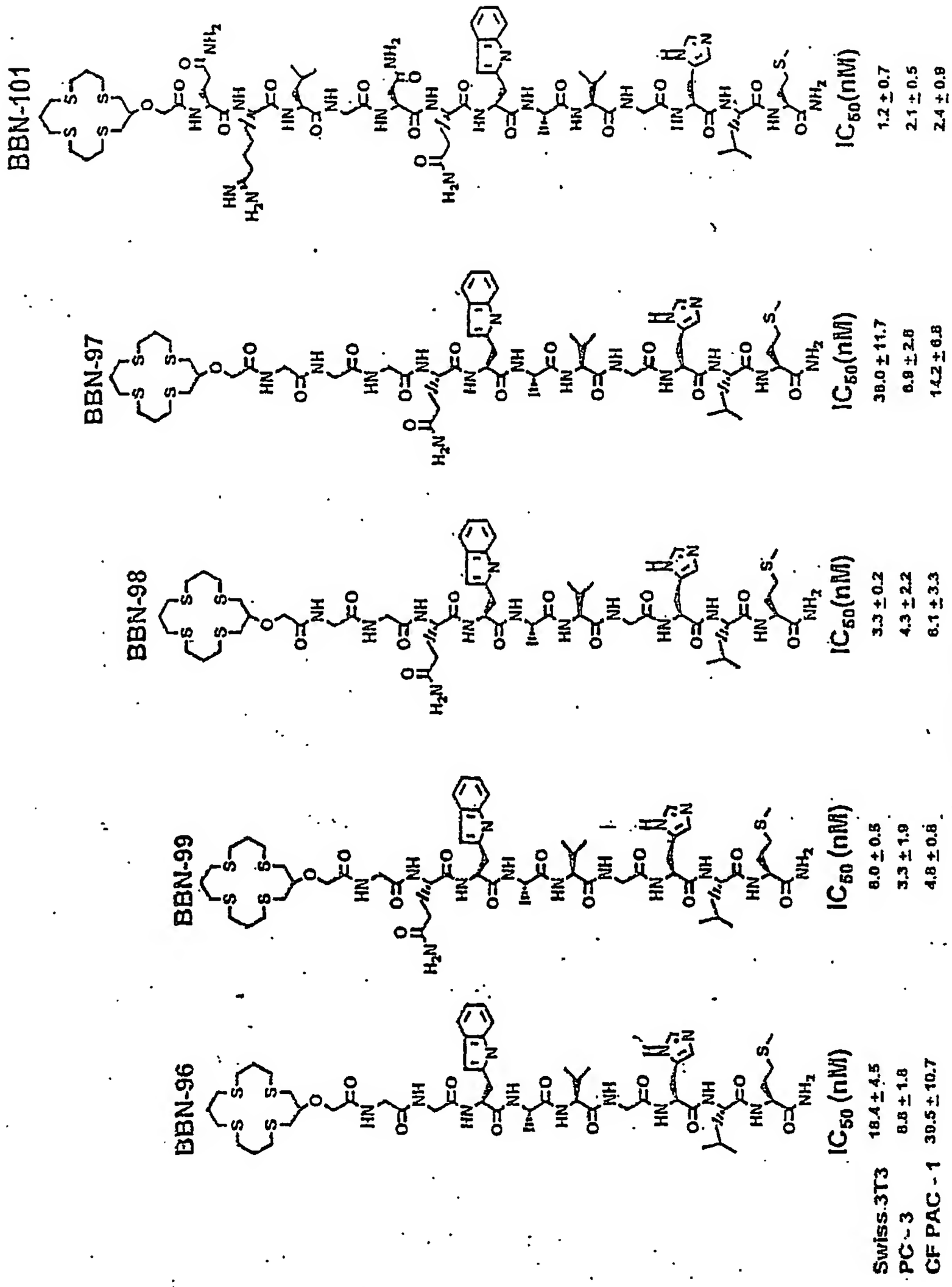

BBN-101
IC50(nM)
1.2 ± 0.7
2.1 ± 0.5
2.4 ± 0.9
BBN-97
IC50(nM)
38.0 ± 11.7
6.9 ± 2.6
14.2 ± 6.8
BBN-98
IC50(nM)
3.3 ± 0.2
4.3 ± 2.2
6.1 ± 3.3
BBN-99
IC50 (nM)
8.0 ± 0.8
3.3 ± 1.9
4.8 ± 0.8
BBN-96
IC50 (nM)
Swiss 3T3 18.4 ± 4.5
PC-3 8.8 ± 1.8
GF PAC - 1 39.5 ± 10.7

FIGURE 19

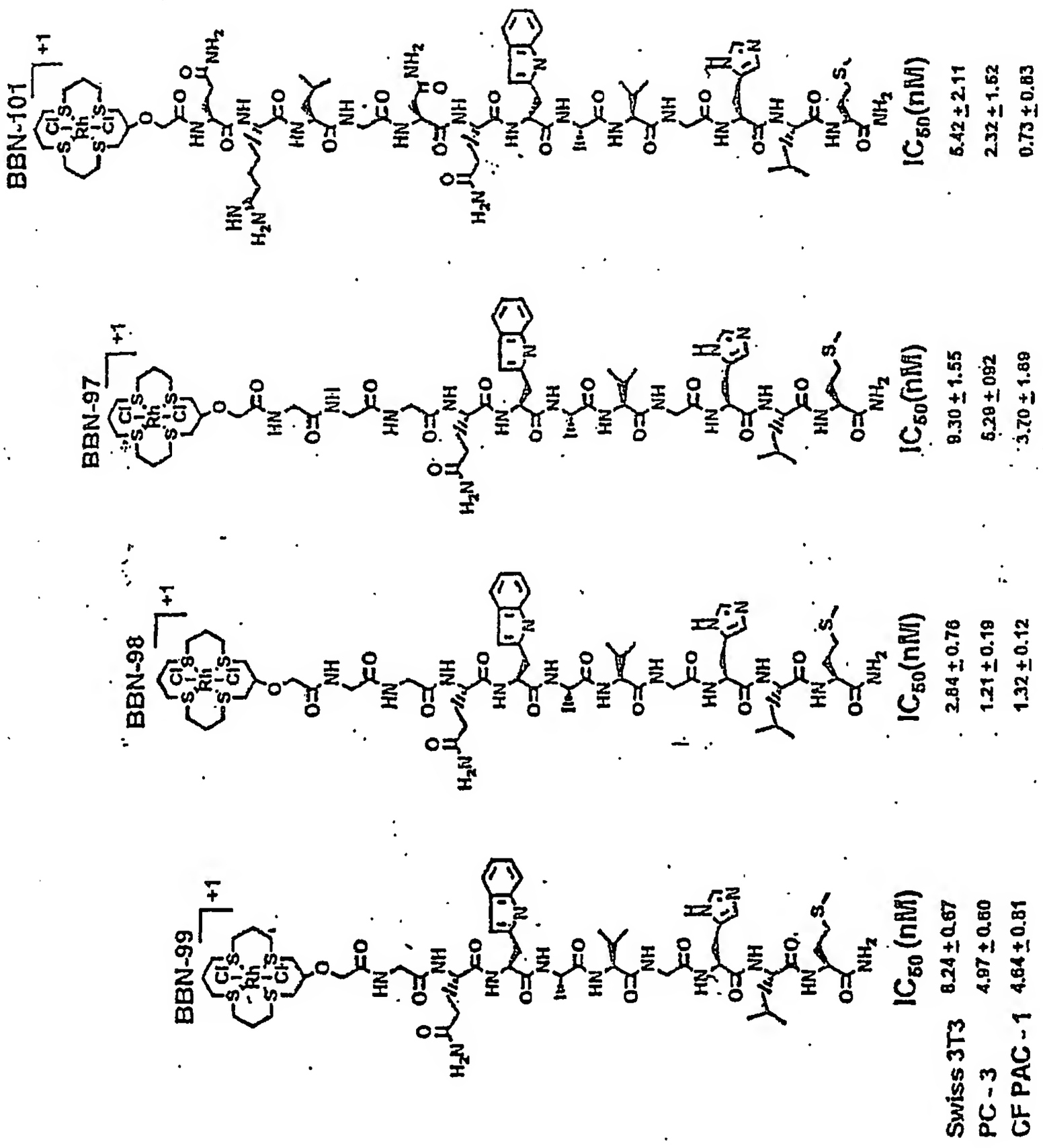
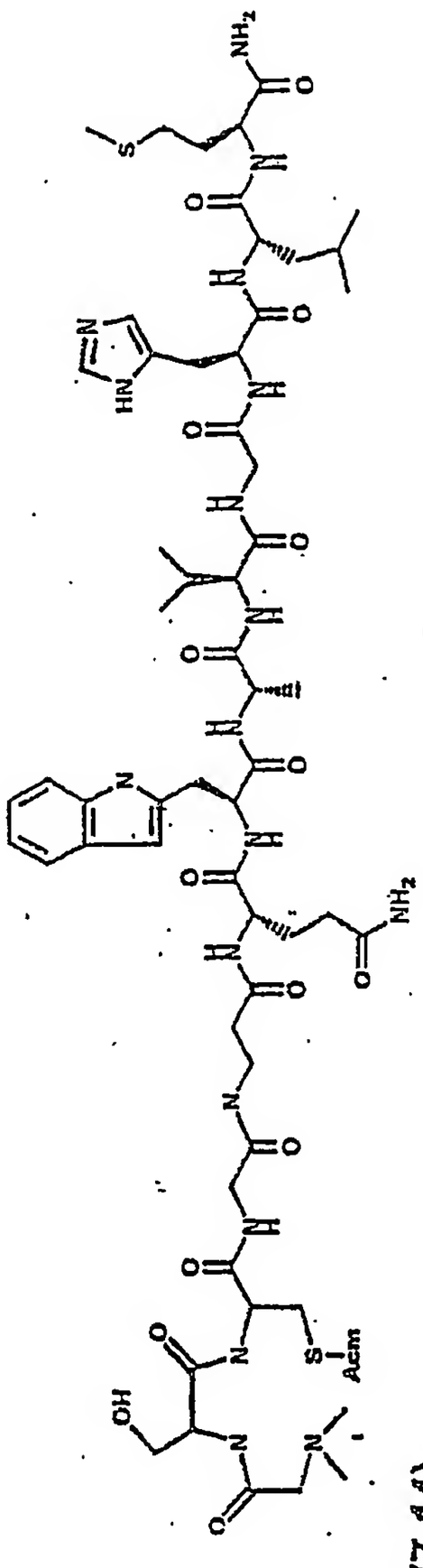
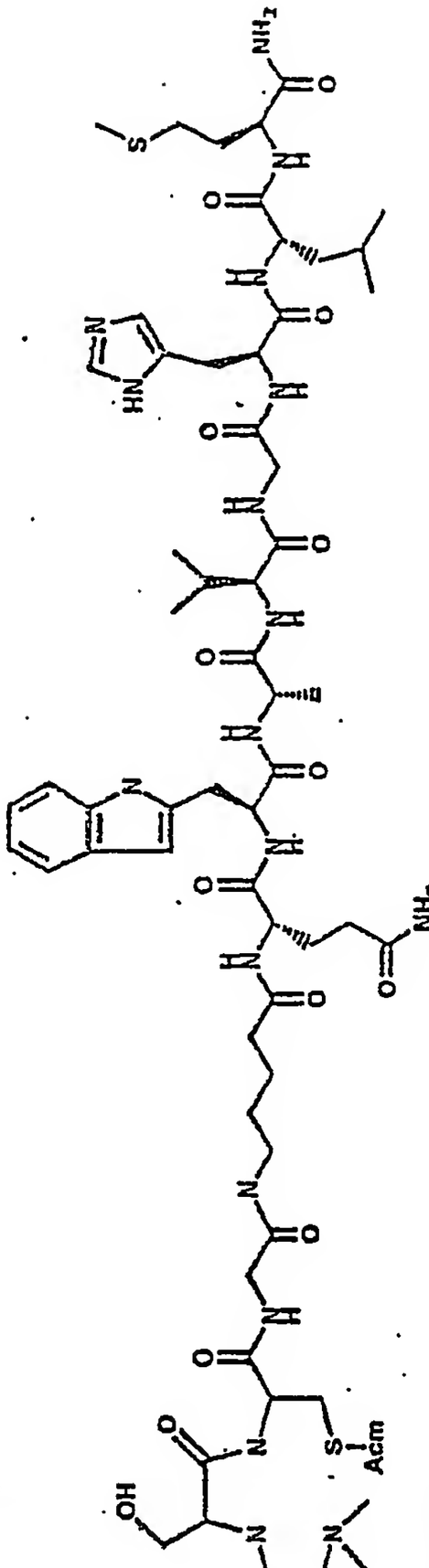

BBN-101
+1
IC50(nM)
5.42 ± 2.11
2.32 ± 1.52
0.73 ± 0.83
BBN-97
+1
IC50(nM)
9.30 ± 1.55
5.29 ± 092
3.70 ± 1.89
BBN-98
+1
IC50(nM)
2.84 ± 0.76
1.21 ± 0.19
1.32 ± 0.12
BBN-99
+1
IC50 (nM)
Swiss 3T3 8.24 ± 0.67
PC - 3 4.97 ± 0.60
CF PAC - 1 4.84 ± 0.81

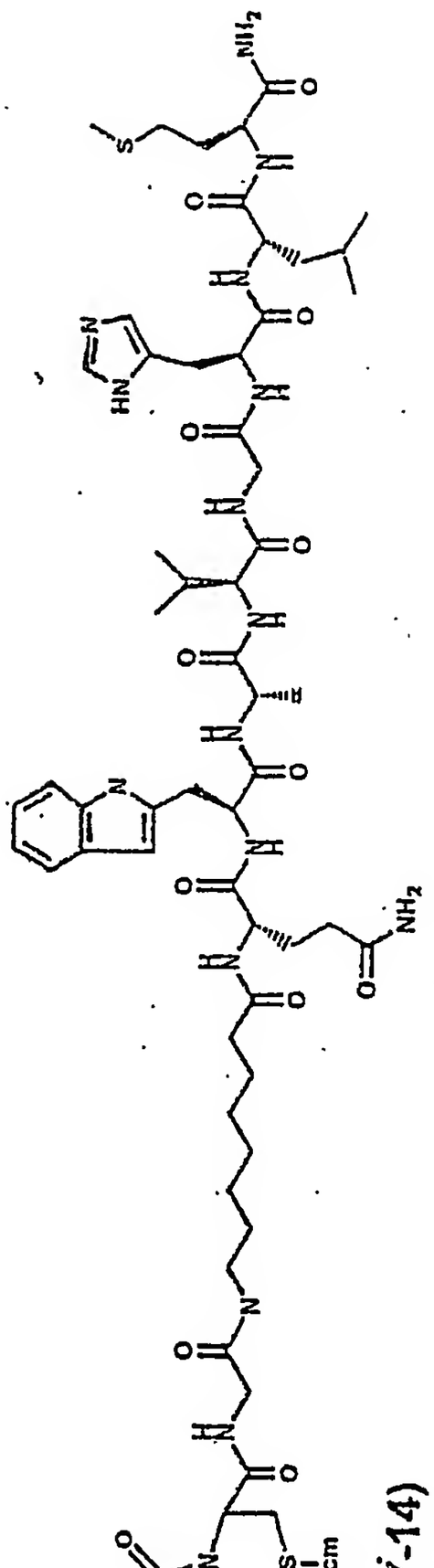
FIGURE 20



RP414-3C-BBN(7-14)



RP414-5C-BBN(7-14)



RP414-8C-BBN(7-14)

FIGURE 21

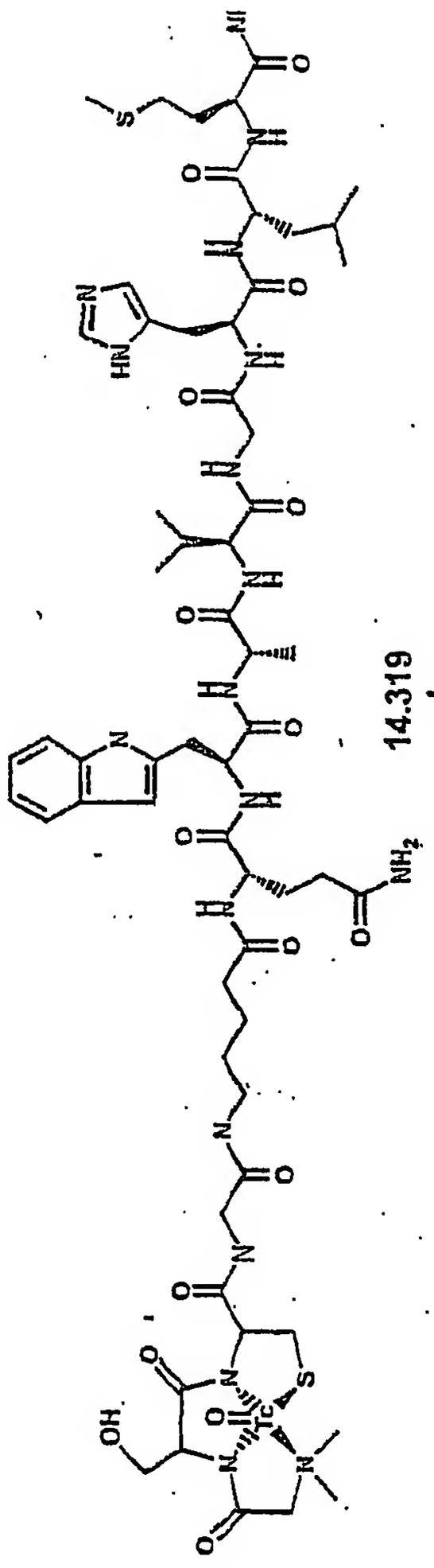


Figure 22

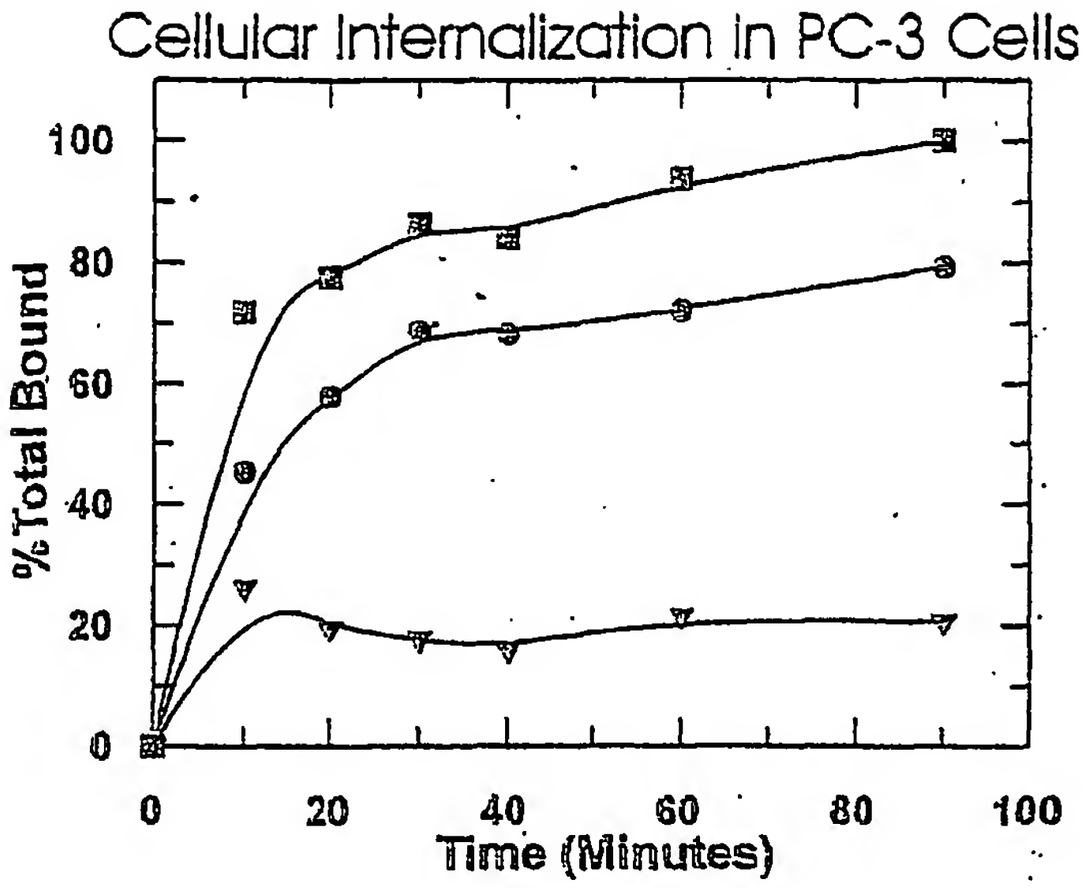

Cellular Internalization in PC-3 Cells
% Total Bound
100
80
60
40
20
0
0
20
40
60
80
100
Time (Minutes)

Figure 23

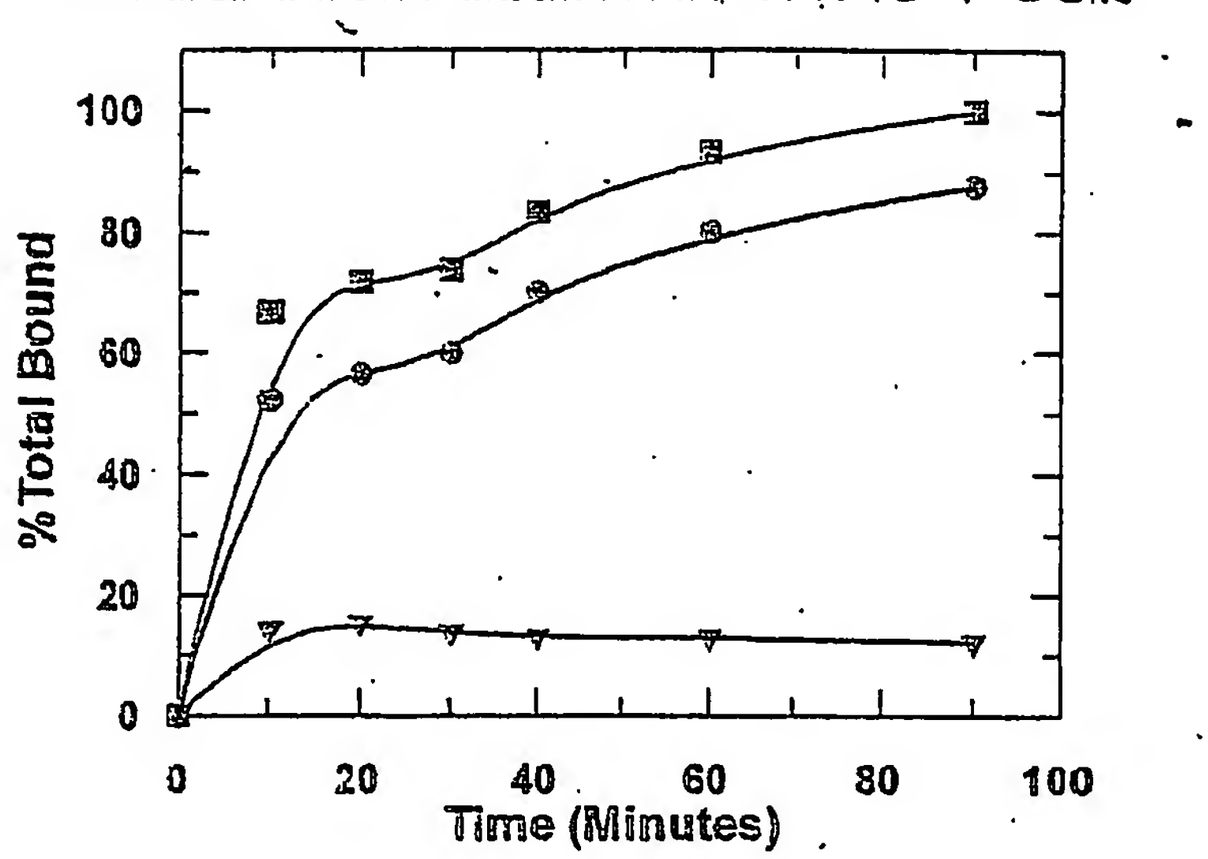

Cellular Internalization in CFPAC-1 Cells
% Total Bound
0
20
40
60
80
100
0
20
40
60
80
100
Time (Minutes)

Figure 24

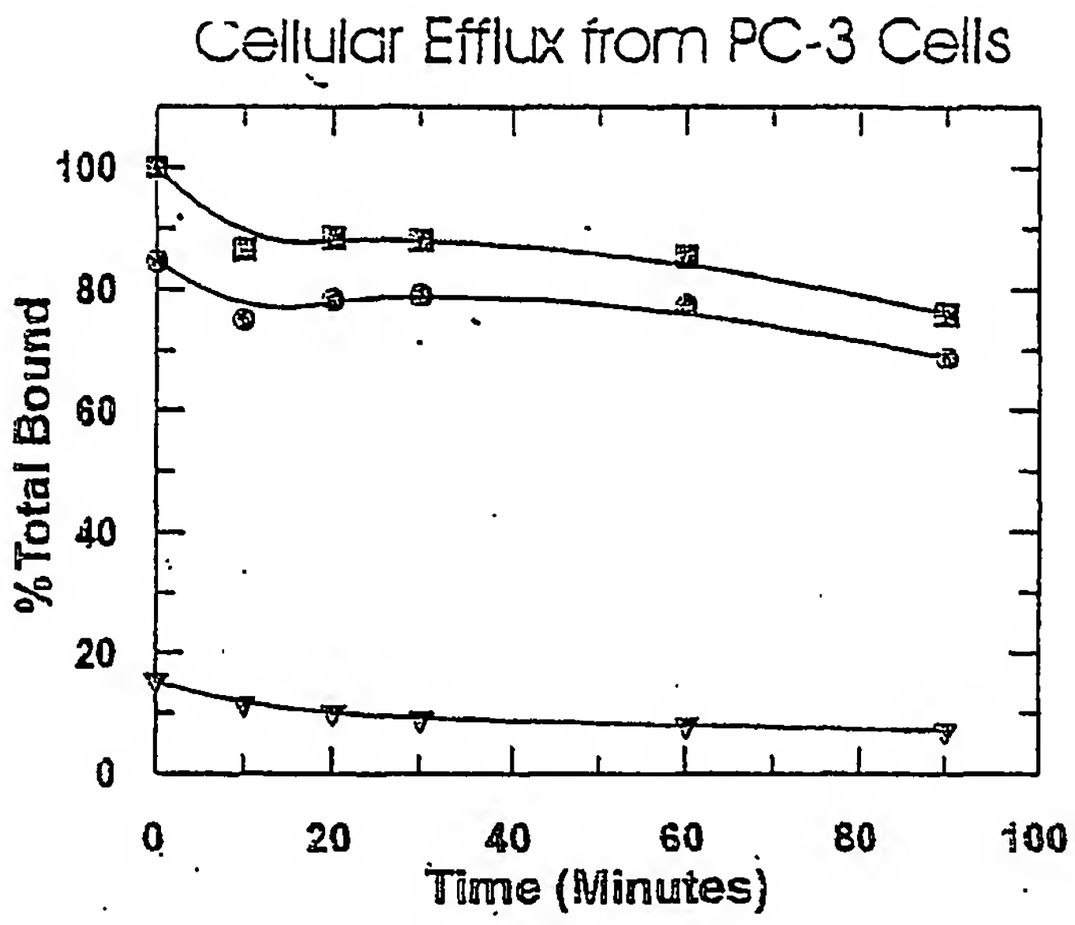

Cellular Efflux from PC-3 Cells
% Total Bound
100
80
60
40
20
0
0
20
40
60
80
100
Time (Minutes)

Figure 25

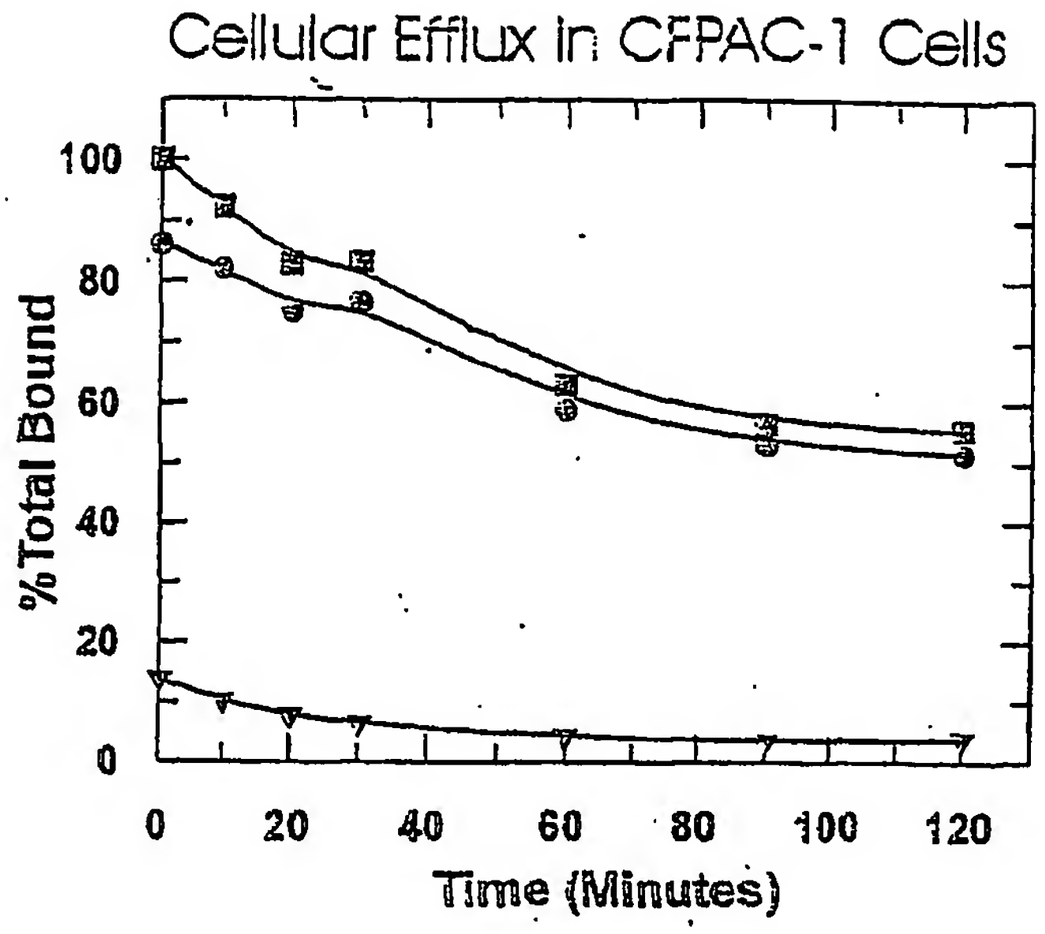
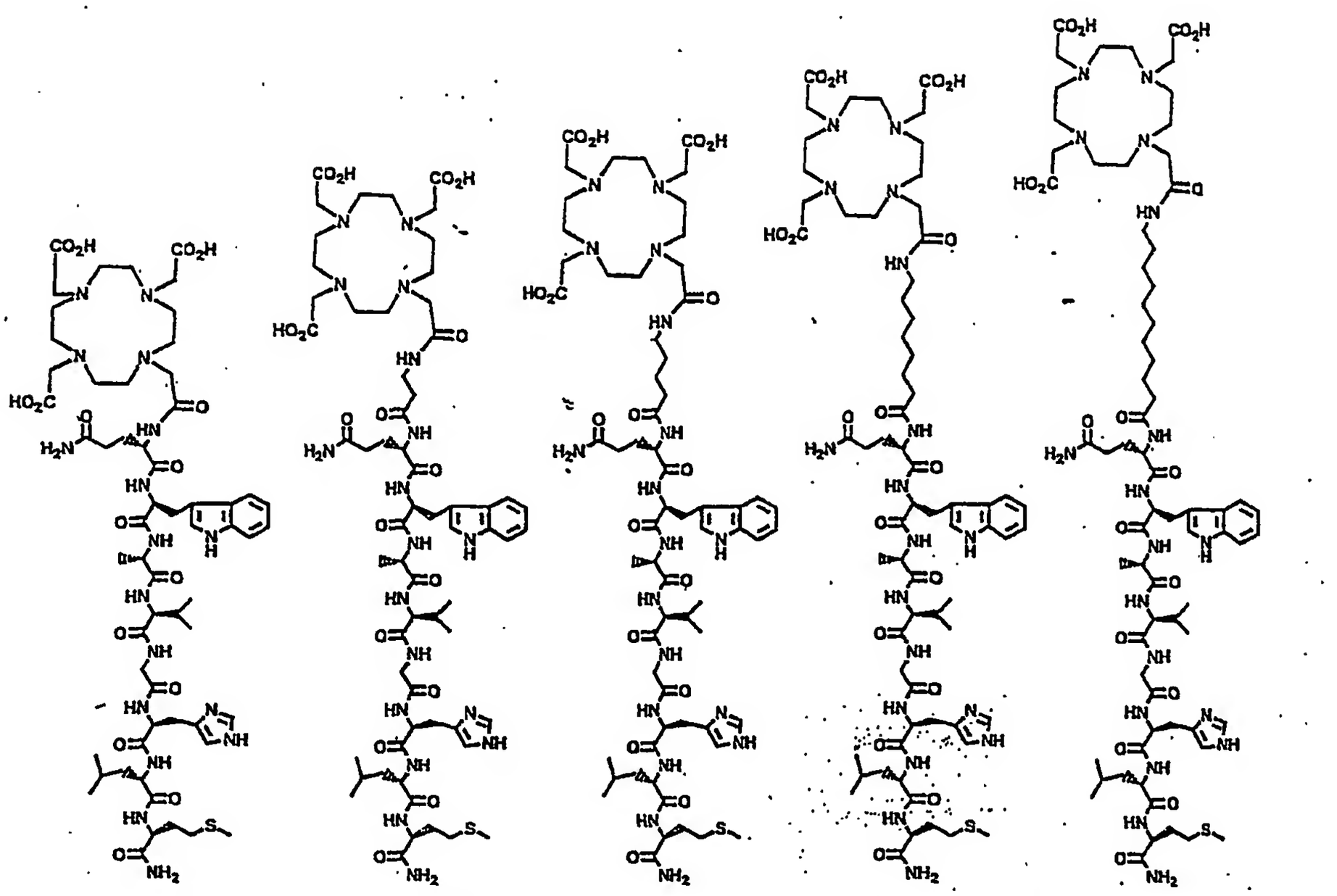

Cellular Efflux in CFPAC-1 Cells
% Total Bound
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (Minutes)

Figure 26



DOTA-0-BBN(7-14)NH_2 DOTA-βAla-BBN(7-14)NH_2 DOTA-5Ava-BBN(7-14)NH_2 DOTA-8Aoc-BBN(7-14)NH_2 DOTA-11Aun-BBN(7-14)NH_2

FIGURE 27

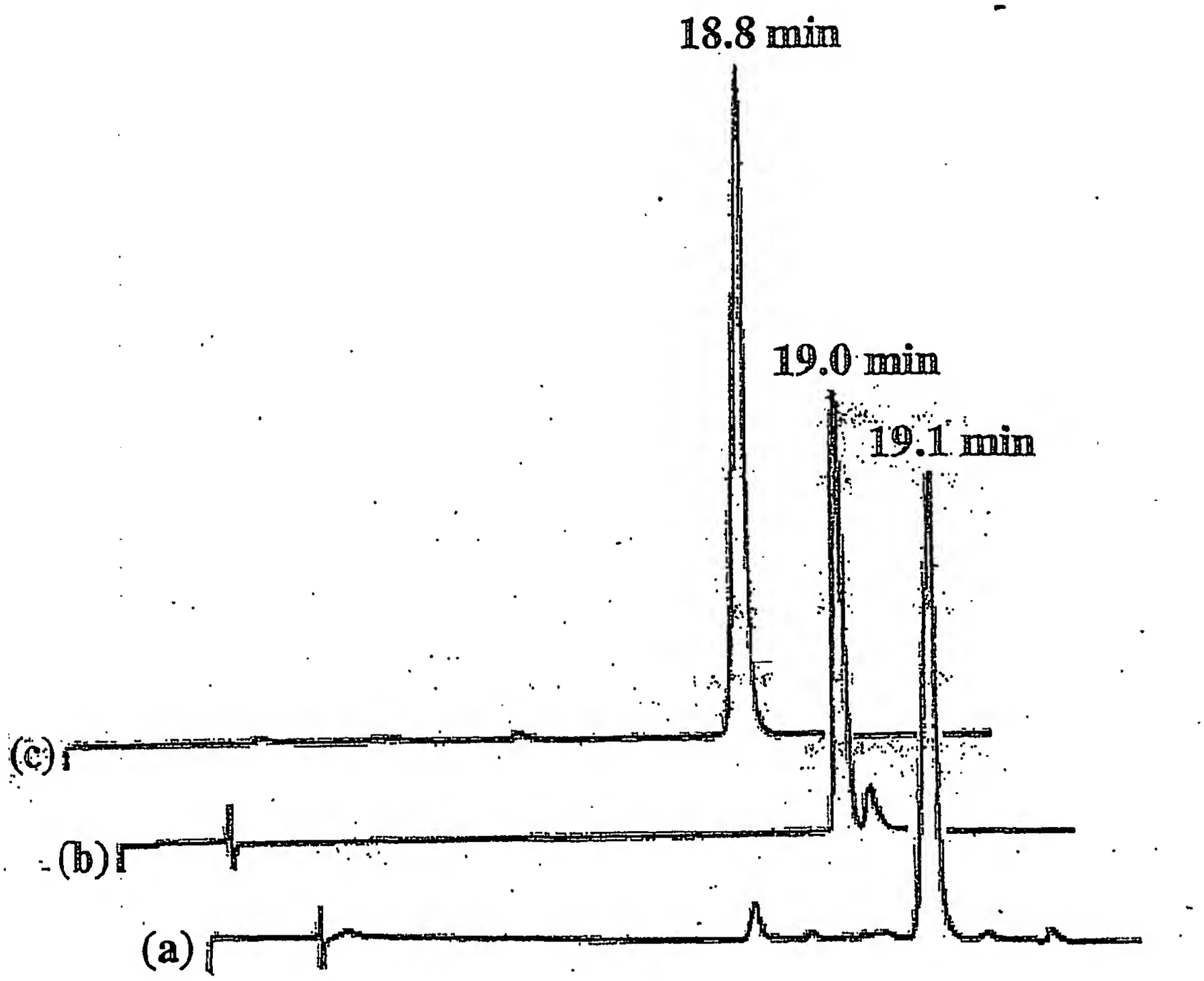


FIGURE 28

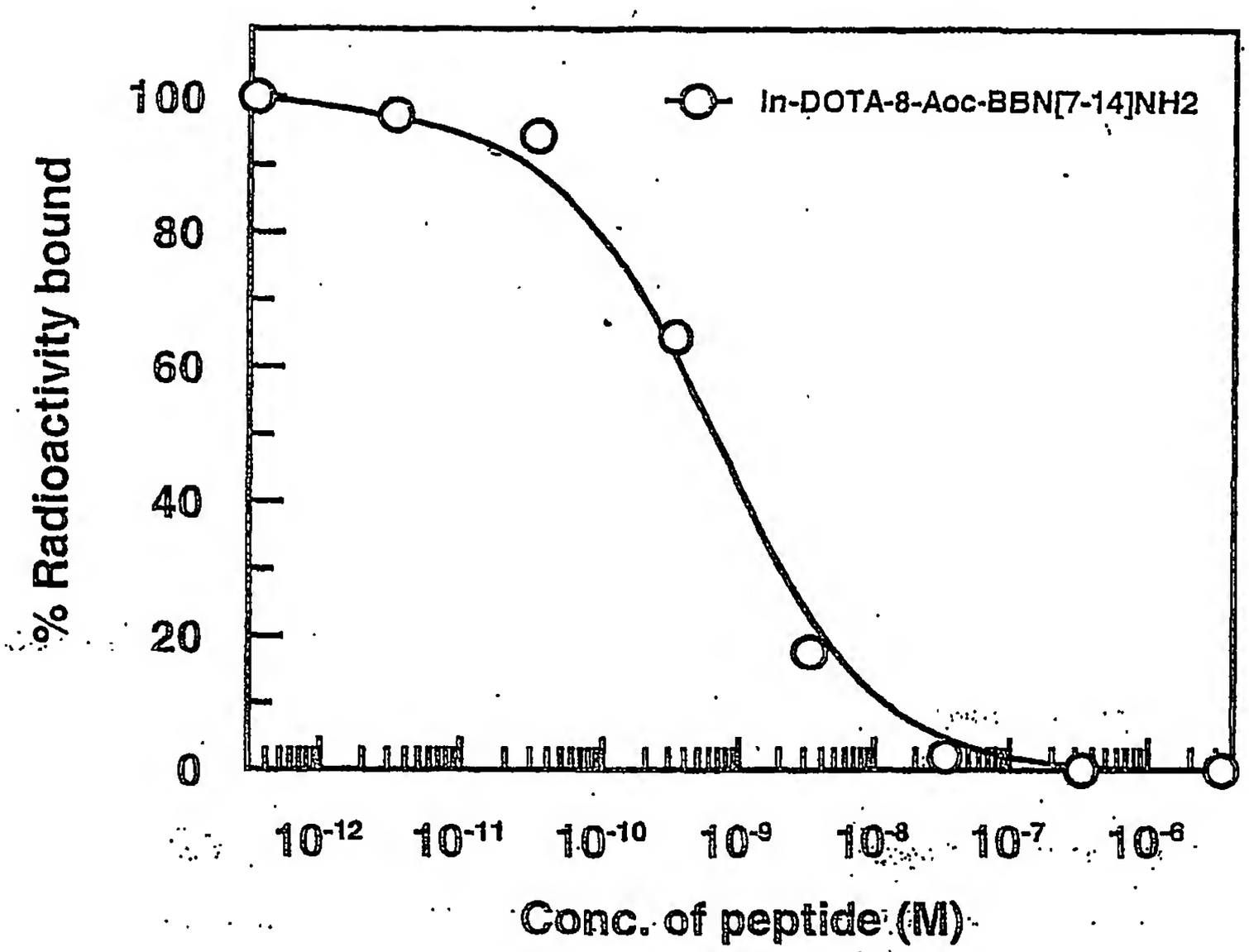

In-DOTA-8-Aoc-BBN[7-14]NH2
% Radioactivity bound
100
80
60
40
20
0
10^{-12}
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
Conc. of peptide (M)

FIGURE 29

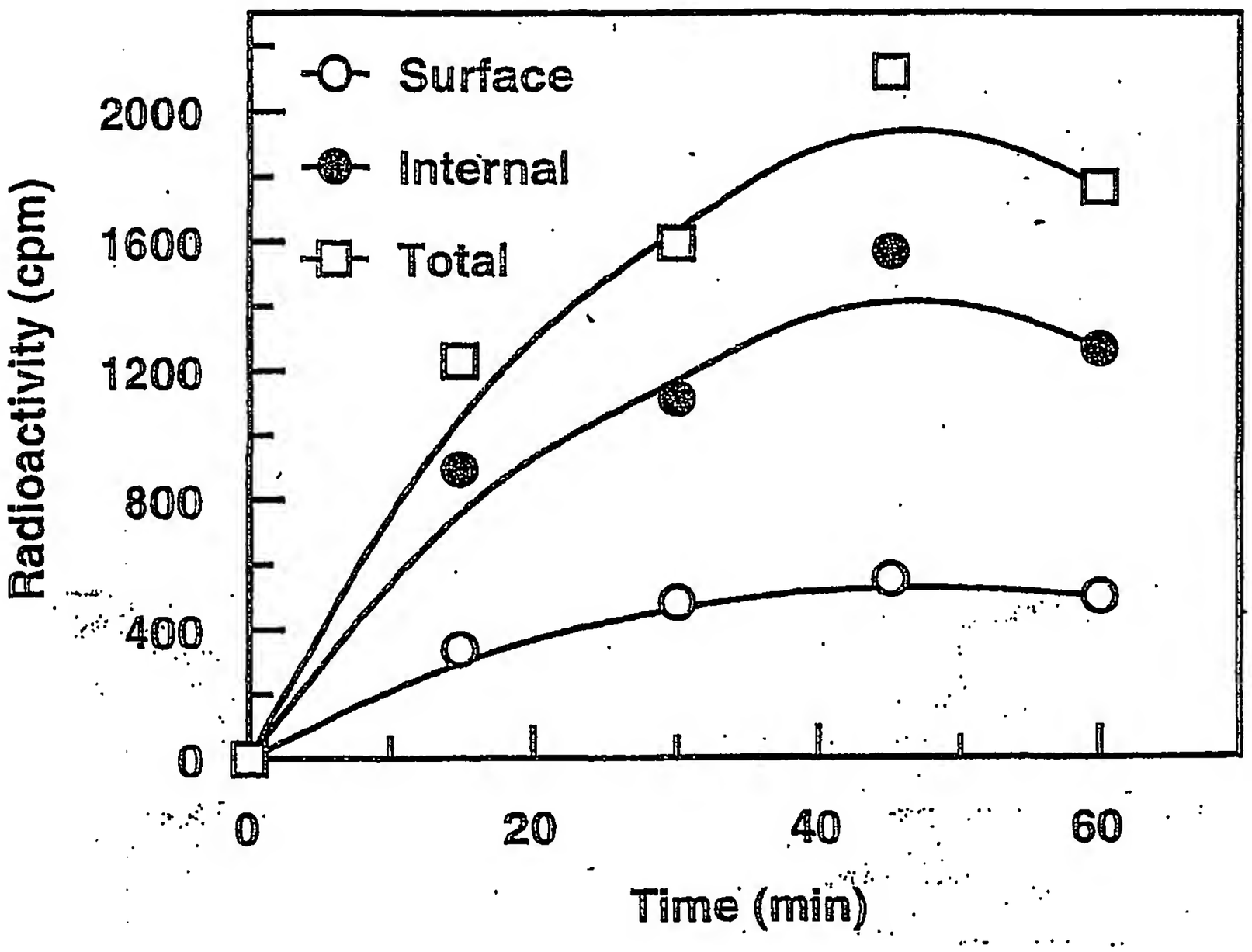

Surface
Internal
Total
2000
1600
1200
800
400
0
Radioactivity (cpm)
0
20
40
60
Time (min)

FIGURE 30

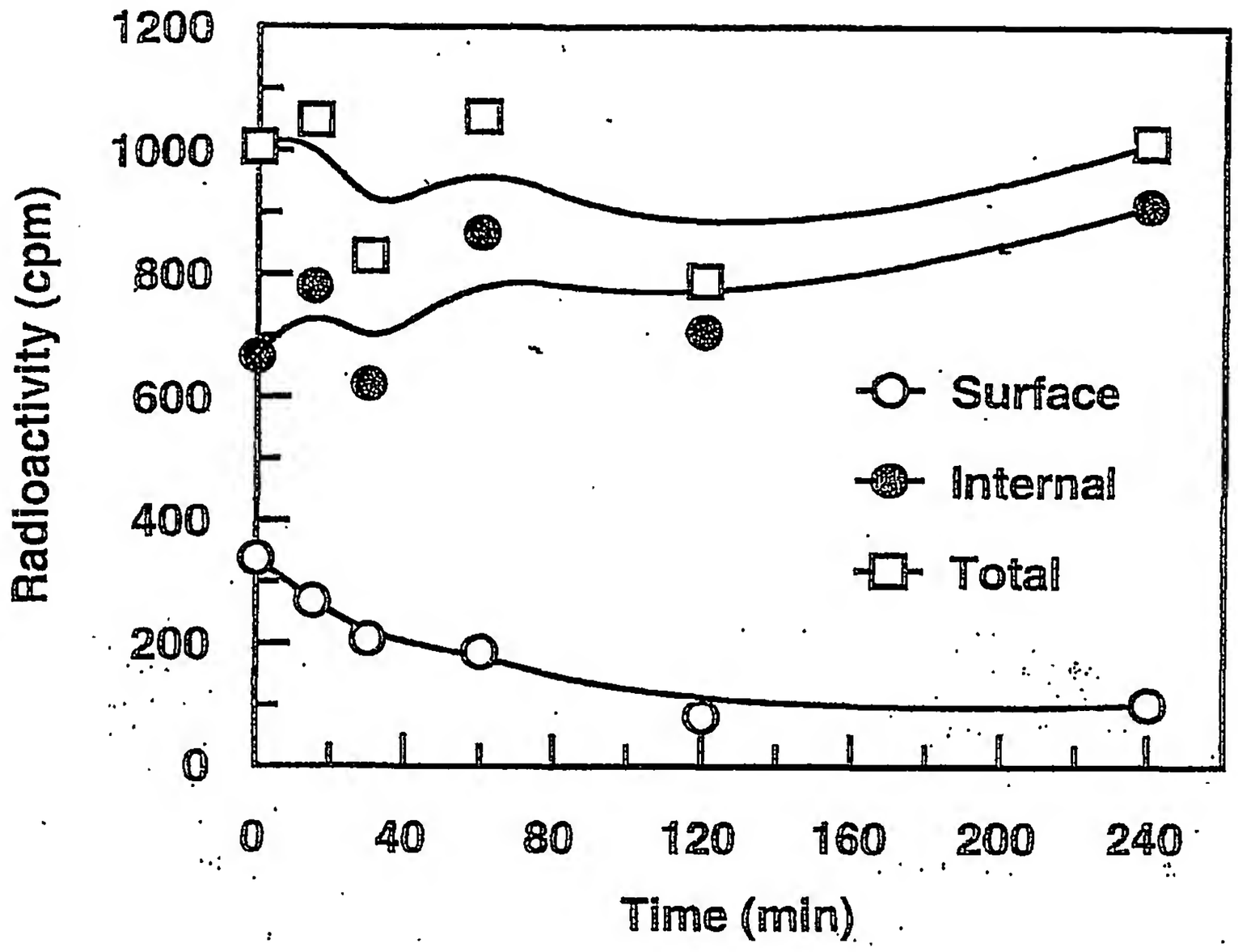

1200
1000
800
600
400
200
0
Radioactivity (cpm)
0
40
80
120
160
200
240
Time (min)
Surface
Internal
Total

FIGURE 31

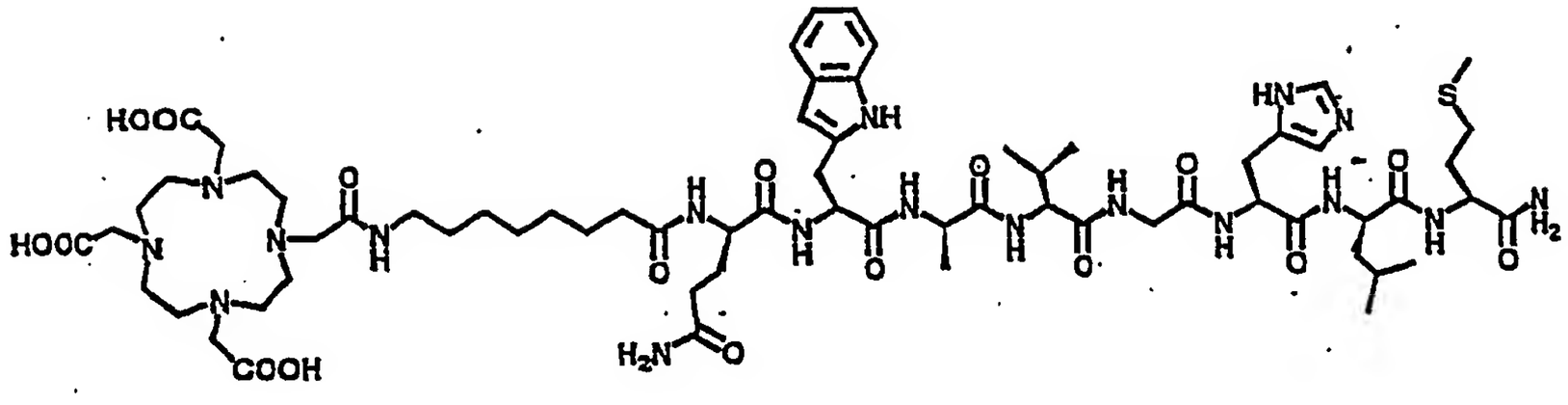


Figure 32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/13840

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 51/00; A61M 36/14
US CL : 424/1.69
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/1.69

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
BIOSIS, REGISTRY, MEDLINE, USPATFUL, EMBASE, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,834,433 A (KRSTENANSKY) 10 November 1998 (10.11.1998), see entire document, especially, abstract and columns 2-3, lines 50-68 and 1-15.	1-61
Y	HEPPELER, A. et al. Receptor Targeting for Tumor Localization and Therapy with Radiopeptides. Current Medicinal Chemistry. 2000, Vol. 7, pages 971-994, especially, abstract; pages 984 and 988.	1-61

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 June 2002 (18.06.2002)	22 JUL 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer D. L. Jones Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)